without loss of affinity or activity to produce A-71623 (10), which binds to the pancreatic receptor with an IC_{50} of 3.7 nM and to the cortical receptor with an IC_{50} of 4500 nM, thus making the compound greater than 1000-fold selective for the CCK-A receptor. A-71623 functioned as a full agonist in potentiating amylase release with an EC_{50} of 0.39 nM and exhibited a biphasic dose–response curve similar to that of CCK-8.[17] The response was effectively blocked by the CCK-A selective antagonist MK-329. On the basis of these results, A-71623 was selected as the prototypic, tetrapeptide-based CCK-A receptor agonist and is presently undergoing evaluation in additional biological assays to establish the therapeutic potential of these compounds.

Thus, we have demonstrated that a tetrapeptide can bind potently to the CCK-A receptor and elicit full agonist activity. We are currently conducting modeling studies to establish how the key structural elements of the tetrapeptide series map against the sulfated octa- and heptapeptides as well as with the various classes of CCK-A receptor antagonists. The results of these investigations along with further details concerning the development of this series will be reported.

(17) The percent maximal response for A-71623 in the amylase assay at 10^{-8} M was 100%; at 3×10^{-8} M, 85%; at 10^{-7} M, 80%; and at 10^{-6} M, 60%. For CCK-8, the percent maximal response at 10^{-9} M was 100%; at 10^{-8} M, 72%; and at 10^{-7} M, 60%. A detailed characterization regarding this aspect of amylase secretion, as well as other biological properties of these analogues, will be addressed in a separate paper submitted for publication by Lin, C. W. et al.

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Phosphorus-Containing Inhibitors of HMG-CoA Reductase. 1. 4-[(2-Arylethyl)hydroxyphosphinyl]-3-hydroxybutanoic Acids: A New Class of Cell-Selective Inhibitors of Cholesterol Biosynthesis

Herein we report a rationale for design and synthesis of a new class of hydroxyphosphinyl-containing 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase) inhibitors of general structure **3** which are as effective in inhibiting cholesterol biosynthesis in vitro and in vivo as compounds with proven therapeutic efficacy (e.g., **1a–c**[1]) as hypocholesterolemic agents.[2] In vitro inhibition of cholesterol biosynthesis in whole cells indicates that these compounds exhibit a remarkably high degree of selectivity for hepatic cells compared to nonhepatic cell types.

On a molecular level, it has been demonstrated[3] that the 3,5-dihydroxyheptanoic acid side chain of the fungal metabolites **1** interacts with the 3-hydroxy-3-methylglutaryl (HMG) binding domain of the enzyme's active site. It has been postulated[3] that the tight binding of reductase inhibitors such as **1** is the result of the compounds ability to simultaneously interact with the HMG binding domain of the enzyme and an adjacent hydrophobic pocket which is not utilized in substrate binding. Previous synthetic studies have focused on replacing the highly functionalized decalin nucleus of the fungal metabolites by a variety of aromatic and heteroaromatic nuclei (e.g., **2**).[4] The design of the hydroxyphosphinyl-containing inhibitors was based on mechanistic consideration of the enzymatic reduction of HMG-CoA by HMG-CoA reductase. Kinetic studies have shown[5] that the enzymatic reaction follows the general chemical mechanism postulated for dehydrogenase catalysis in which a group on the enzyme acts as an acid–base catalyst to assist in direct transfer of a hydride ion between nucleotide and substrate. The pK_a of this catalytic group is dependent on whether reduced or oxidized cofactor (NADPH or $NADP^+$, respectively) is bound at the active site. The dihydroxyheptanoic acid inhibitors **1** and **2** apparently owe their inhibitory activity to their ability to mimic the half-reduced substrate mevaldate hemithioacetal. Therefore, by analogy, the 5-hydroxyl group of **1** or **2** must interact with the unprotonated form of this catalytic group. The hydroxyphosphinyl-containing inhibitors **3**, as the corresponding phosphinate anions, were designed to ion pair with the protonated form of the catalytic group[6] which normally serves to activate substrate carbonyl groups toward reduction.

With the goal of developing a general synthetic route to the hydroxyphosphinyl-containing compounds **3** which would allow maximum flexibility with respect to the "hydrophobic anchor" portion of the inhibitors, we envisioned the retrosynthetic disconnection shown in Figure 1. The required phosphonochloridate **5** in homochiral form[7] was prepared by a multistep route from isoascorbic acid (**6**) via known[8] bromohydridin ester **7** by the route

(1) Compounds **1a,b** are shown in their biologically active dihydroxy forms; compactin and lovastatin are the δ-lactone forms of **1a** and **1b**, respectively.

(2) Hoeg, J. M.; Brewer, H. B. *J. Am. Med. Assoc.* **1987**, *258*, 3532.

(3) Abeles, R. H.; Nakamura, C. E. *Biochemistry* **1985**, *24*, 1364.

(4) (a) Stokker, G. E.; Hoffman, W. F.; Alberts, A. W.; Cragoe, E. J.; 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. (b) Hoffman, W. F.; Alberts, A. W.; Cragoe, E. J.; Deana, A. A.; Evans, B. E.; Gilfillan, J. L.; Gould, N. P.; Huff, J. W.; Novello, F. C.; Prugh, J. D.; Rittle, K. E.; Smith, R. L.; Stokker, G. E.; Willard, A. K. *J. Med. Chem.* **1986**, *29*, 159. (c) Stokker, G. E.; Alberts, A. W.; Anderson, P. S.; Cragoe, E. J.; Deana, A. A.; Gilfillan, J. L.; Hirshfield, J.; Holtz, W. J.; Hoffman, W. F.; Huff, J. W.; Lee, T. J.; Novello, F. C.; Prugh, J. D.; Rooney, C. S.; Smith, R. L.; Willard, A. K. *J. Med. Chem.* **1986**, *29*, 170. (d) Stokker, G. E.; Alberts, A. W.; Gilfillan, J. L.; Huff, J. W.; Smith, R. L. *J. Med. Chem.* **1986**, *29*, 852. (e) Balasubramanian, N.; Brown, P. L.; Catt, J. D.; Han, W. T.; Parker, R. A.; Sit, S. Y.; Wright, J. J. *J. Med. Chem.* **1989**, *32*, 2038. (f) Roth, B. D.; Ortwine, D. F.; Hoefle, M. L.; Stratton, C. D.; Sliskovic, D. R.; Wilson, M. W.; Newton, R. S. *J. Med. Chem.* **1990**, *33*, 21. (g) Sliskovic D. R.; Roth, B. D.; Hoefle, M. L.; Wilson, M. W.; Newton, R. S. *J. Med. Chem.* **1990**, *33*, 31. (h) Beck, G.; Kesseler, K.; Baader, E.; Bartmann, W.; Bergmann, A.; Granzer, E.; Jendralla, H.; Kerekjarto, B. v.; Krause, R.; Paulus, E.; Schubert, W.; Wess, G. *J. Med. Chem.* **1990**, *33*, 52. (i) Jendralla, H.; Baader, E.; Bartmann, W.; Beck, G.; Bergmann, A.; Granzer, E.; Kerekjarto, B. v.; Kesseler, K.; Krause, R.; Schubert, W.; Wess, G. *J. Med. Chem.* **1990**, *33*, 61.

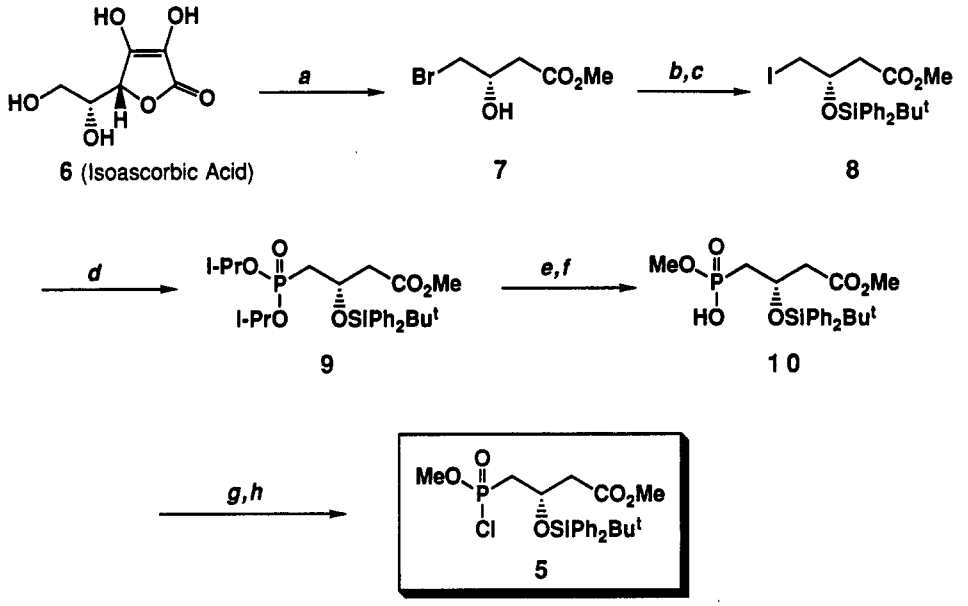
(5) (a) Qureshi, N.; Dugan, R. E.; Cleland, W. W.; Porter, J. W. *Biochemistry* **1976**, *15*, 4185, 4191. (b) Veloso, D.; Cleland, W. W.; Porter, J. W. *Biochemistry* **1981**, *20*, 887.

(6) Workers at SmithKline Beecham have recently reported a kinetic study of several slow-binding inhibitors of HMGR including phosphinate **3n**. From pH studies they concluded that **3n** binds to a protonated form of the enzyme while the dihydroxy compounds bind to a deprotonated form of the enzyme. Louis-Flamberg, P.; Peishoff, C. E.; Bryan, D. L.; Leber, J.; Elliott, J. D.; Metcalf, B. W.; Mayer, R. J. *Biochemistry* **1990**, *29*, 4115.

(7) Compounds **5**, **14**, and **15**, while enantiomerically pure, are mixtures of diastereomers at phosphorus. Compounds **3**, unless otherwise indicated, are single, homochiral diastereomers.

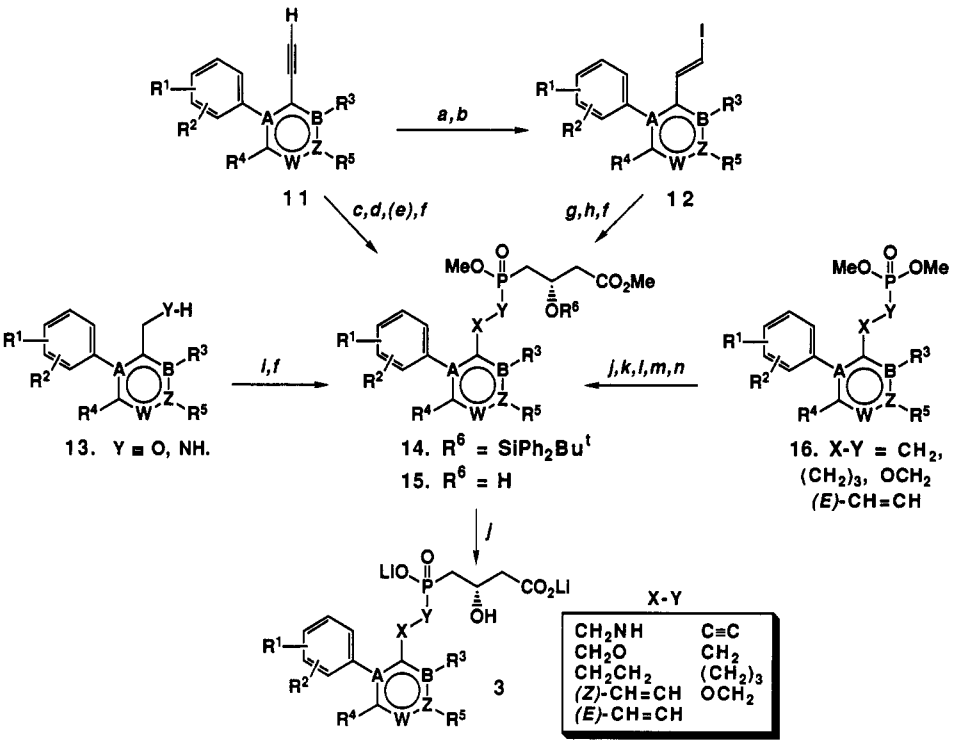
0022-2623/90/1833-2952$02.50/0

Scheme I[a]



[a] (a) See ref 8; (b) t-$BuPh_2SiCl$, DMAP, imidazole, DMF; (c) NaI, MEK, reflux (74% from 7); (d) triisopropyl phosphite, 150–160 °C, 16 h (75%); (e) TMSBr, BSTFA, CH_2Cl_2; (f) MeOH, DCC, pyridine, 20 h (isolated as its dicyclohexylamine salt, 77%); (g) $TMSNEt_2$, CH_3Cl_2; (h) oxalyl chloride, DMF (0.05 equiv), CH_2Cl_2.

Scheme II[a]



[a] (a) n-Bu_3SnH, AIBN, 140 °C; (b) I_2, Et_2O; (c) n-BuLi, THF, −78 °C; (d) **5**, THF, −78 °C; (e) H_2, Pd–C, MeOH; (f) n-Bu_4NF (3 equiv), AcOH (4 equiv), THF; (g) t-BuLi, THF, −78 °C; (h) **5**, THF, −100 °C; (i) **5**, pyridine, room temperature; (j) 1 N LiOH, dioxane; (k) $TMSNEt_2$, CH_2Cl_2; (l) oxalyl chloride, catalytic DMF, CH_2Cl_2; (m) $CH_3COCH_2CO_2Me$, NaH, n-BuLi, −78 °C; (n) $NaBH_4$, EtOH.

outlined in Scheme I.

For the synthesis of the phosphonic monoester **3** (X–Y = CH_2O) and phosphonamide **3** (X–Y = CH_2NH) based inhibitors (Scheme II), phosphonochloridate **5** was condensed with the appropriate alcohols **13** (Y = O) or amines **13** (Y = NH) to give, after silyl ether cleavage, the target compounds as the corresponding dimethyl esters **15** (X–Y = CH_2O or CH_2NH). Methyl ester hydrolysis proceeded smoothly in the case of the oxygen isosteres to provide the desired phosphonic monoesters **3** (X–Y = CH_2O), isolated as their dilithium salts. In contrast, the corresponding phosphonamides **3** (X–Y = CH_2NH), also prepared by hydrolysis of the corresponding dimethyl esters, proved to be too unstable to isolate in pure form.

One of the routes we have developed for the synthesis of the phosphinic acid based inhibitors utilizes the con-

(8) (a) Bock, K.; Lundt, I.; Pedersen, C. *Acta Chem. Scand., B.* **1983**, *37*, 341. (b) Isbell, H. S.; Frush, H. L. *Carbohydr. Res.* **1979**, *72*, 301.

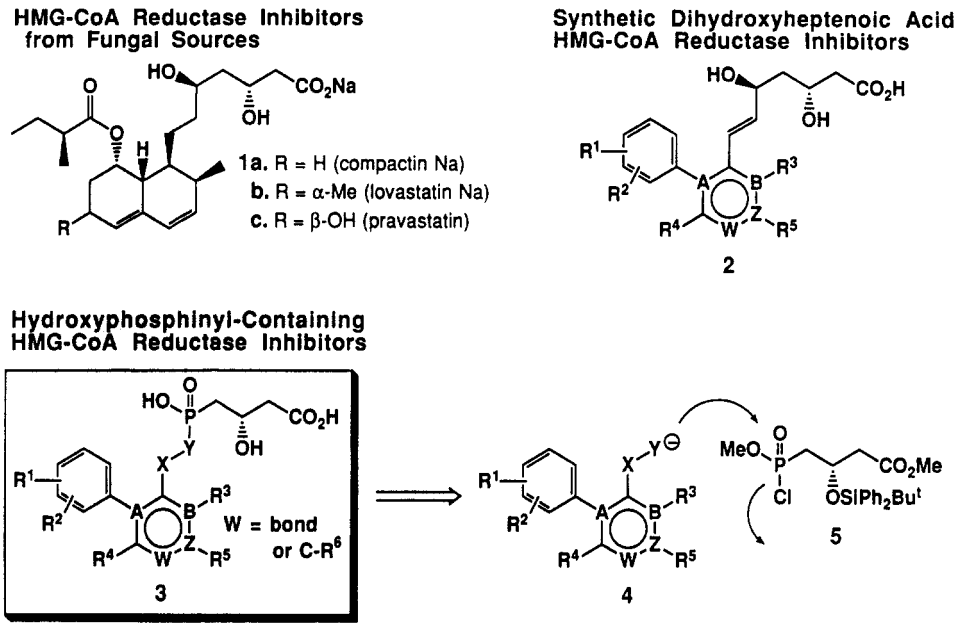


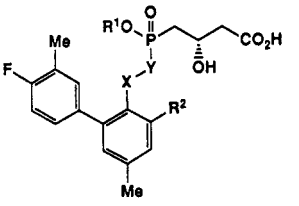
Figure 1.

densation between phosphonochloridate **5** and acetylenic anions. Condensation of the lithium anion of acetylenes **11**[9] with phosphonochloridate **5** proceeded quite smoothly at −78 °C to give the desired acetylenic phosphinates **14** (X–Y = C≡C), typically in yields of 70–90%. Acetylenic phosphinates **14** (X–Y = C≡C) are versatile intermediates and were used to prepare inhibitors **3** with saturated (X–Y = CH_2CH_2), acetylenic (X–Y = C≡C), and cis-unsaturated (X–Y = (*Z*)-CH=CH) linkages (see Scheme II).

The terminal acetylenes **11** also serve as precursors of the corresponding *trans*-vinylphosphinates. Hydrostannation of acetylenes **11** under free-radical conditions[10] followed by treatment with iodine provided the corresponding *trans*-vinyl iodides **12**. Metalation with *t*-BuLi at −78 °C followed by condensation with phosphonochloridate **5** at −100 °C afforded the desired *trans*-phosphinates **14** (X–Y = (*E*)-CH=CH). Deprotection using our standard protocol gave the *trans*-vinylphosphinic acids **3** (X–Y = (*E*)-CH=CH).

The target phosphinic acid based inhibitors can also be prepared in racemic form by a route which utilizes an alternative carbon–phosphorus bond disconnection. Condensation of the phosphonochloridates derived from dimethyl phosphonates **16**[11] with the dianion of methyl acetoacetate[12] (−78 °C, THF) followed by reduction of the resulting β-ketophosphinate with sodium borohydride in ethanol gave diesters **15** in racemic form. This route proved to be particularly useful as an alternative synthesis of the *trans*-vinylphosphinic acids **3** (X–Y = (*E*)-CH=CH).

Table I



no.	X–Y	R^1	R^2	reductase I_{50}, μM
3a-Me	CH_2NH	Me	Me	70.5
3b-Me	CH_2O	Me	Me	21.6
3c-Me	CH_2CH_2	Me	Me	4.6
3b	CH_2O	H	Me	0.162
3c	CH_2CH_2	H	Me	0.015
3d	(*E*)-CH=CH	H	Me	0.0067
3e	(*Z*)-CH=CH	H	Me	3.0
3f	C≡C	H	Me	0.297
3g	CH_2[a]	H	Me	89.3
3h	$(CH_2)_3$[a]	H	Me	150
3i	OCH_2[a]	H	Me	4.5
3j	CH_2O	H	*i*-Pr	0.0012
3k	CH_2CH_2	H	*i*-Pr	0.0038
3l	C≡C	H	*i*-Pr	0.0085

[a] *R,S* mixture.

For the purposes of our initial investigation, we prepared a series of prototype hydroxyphosphinyl-containing inhibitors **3b–i** based on a substituted biphenyl nucleus[13] which differ only in the linking group X–Y (see Table I). Methyl esters **3a**-Me, **3b**-Me, and **3c**-Me were prepared in order to independently study the effect of heteroatom substitution adjacent to phosphorus on inhibitory activity and to factor out any differences in the liganding ability

(9) Terminal acetylenes **11** were prepared via a one-carbon homologation of the corresponding aldehydes. (a) Corey, E. J.; Fuchs, P. L. *Tetrahedron Lett.* **1972**, 3769. (b) Gilbert, J. C.; Weeraooriya, U. *J. Org. Chem.* **1979**, *44*, 4997. (c) Tanaka, H.; Yamashita, S.; Yamanoue, M.; Torii, S. *J. Org. Chem.* **1989**, *54*, 444. (d) Burton, G.; Elder, J. S.; Fell, S. C. M.; Stachulski, A. V. *Tetrahedron Lett.* **1988**, *29*, 3003. (e) Negishi, E.-i.; King, A. O.; Klima, W. L. *J. Org. Chem.* **1980**, *45*, 2526.

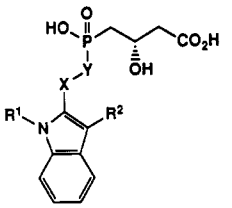
(10) Tolstitkov, G. A.; Maftakhov, M. S.; Danilova, N. A.; Vel'der, Y. L. *Synthesis* **1986**, 496.

(11) Dimethyl phosphonates **16** (X–Y = CH_2 and $(CH_2)_3$) were prepared by Arbuzov reaction of the corresponding bromides with trimethyl phosphite. *trans*-Vinylphosphonates **16** (X–Y = (*E*)-CH=CH) were prepared by treatment of the corresponding aryl aldehydes with dimethyl (lithiomethyl)-phosphonate (THF, −78 °C) followed by acid-catalyzed dehydration of the resulting β-hydroxyphosphonates (*p*-toluenesulfonic acid, benzene, reflux with azeotropic removal of water). Dimethyl phosphonates **16** (X–Y = OCH_2) were prepared by alkylation of the corresponding phenols with dimethyl[[(*p*-tolylsulfonyl)oxy]methyl]phosphonate (NaH, DMF).

(12) Huckin, S. N.; Weiler, L. *Tetrahedron Lett.* **1971**, 4835.

(13) This substituted biphenyl hydrophobic anchor was developed by the Merck group in the dihydroxyheptenoic acid inhibitor series (see ref 4c).

Table II



no.	X-Y	R^1	R^2	reductase I_{50}, μM
3m	CH_2CH_2	*i*-Pr	*p*-FC_6H_4	0.024
3n	(*E*)-CH=CH[a]	*i*-Pr	*p*-FC_6H_4	0.026
3o	C≡C	*i*-Pr	*p*-FC_6H_4	0.006
3p	CH_2CH_2	*p*-FC_6H_4	*i*-Pr	0.120
3q	(*E*)-CH=CH	*p*-FC_6H_4	*i*-Pr	0.0019
3r	C≡C	*p*-FC_6H_4	*i*-Pr	0.0022
3s	CH_2CH_2	*p*-FC_6H_4	Et	0.013
3t	C≡C	*p*-FC_6H_4	Et	0.0875

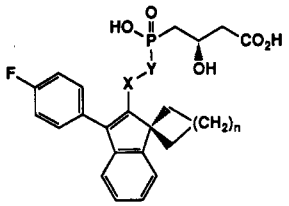
[a] R,S mixture.

of the various hydroxyphosphinyl anions. In the methyl ester series, the most potent analogue was carbon isostere **3c**-Me with a modest I_{50} against rat microsomal reductase[14] of 4.6 μM. As expected on the basis of our binding hypothesis, the corresponding phosphinic acid **3c** was a considerably more potent inhibitor of the enzyme (300-fold), indicating that an ionizable hydroxyphosphinyl function is essential for optimal binding to the enzyme's active site. Shortening or lengthening the two-carbon, saturated linkage of the parent phosphinic acid **3c** results in a dramatic decrease in inhibitory potency. Among the two-carbon unsaturated-linked analogues **3d–f**, *trans*-olefin **3d** was the most active. Introduction of an oxygen atom either adjacent to phosphorus (i.e., **3b**) or adjacent to the aromatic ring (i.e., **3i**) results in a substantial loss of potency relative to the all-carbon isostere **3c**. Replacement of the *o*-methyl substituent in **3b**, **3c**, or **3f** by an isopropyl group (**3j**, **3k**, and **3l**, respectively), results in a dramatic increase in inhibitory potency, especially in the case of the phosphonate (**3j**) (135-fold) and acetylenic (**3l**) (35-fold) analogues.

In agreement with results obtained with dihydroxyheptanoic acid based inhibitors (e.g., **2**), replacement of the central aromatic ring of the biphenyl-based phosphinic acids by either a 3-phenylindole or 1-phenylindole nucleus[15] was compatible with good inhibitory activity (Table II). Within each series, the acetylenic-link indole analogues bearing an *o*-isopropyl substituent **3o** and **3r** were among the most potent inhibitors. Replacement of the isopropyl group in **3r** by an ethyl group (i.e., **3t**) led to a 40-fold decrease in inhibitory potency while the same modification in the saturated-link compound **3p** (i.e., **3s**) resulted in a 9-fold *increase* in activity. The results clearly indicate that the *o*-alkyl substituent of these compounds must be tailored to the nature of the two-carbon linker in order to achieve optimal potency.

Replacement of the indole nucleus by a spiroindene hydrophobic anchor[16] again resulted in an extremely potent series of compounds (Table III). In this case, the acetylenic-link compounds (e.g., **3v**) were equipotent to their *trans*-olefin counterparts (e.g., **3u**). Increasing spiro ring size from five to six membered had little effect on activity (compare **3v** and **3w**). In contrast, spirocyclopropyl analogue **3x** was significantly less active.

Table III



no.	X-Y	*n*	reductase I_{50}, μM
3u	(*E*)-CH=CH[a]	2	0.0021
3v	C≡C	2	0.0028
3w	C≡C	3	0.0030
3x	C≡C	0	0.070

[a] R,S mixture.

Table IV

	I_{50}, μM			in vivo ED_{50}, mg/kg	
no.	reductase	hepatocyte	fibroblast	iv	po
1b	0.004	0.146	0.019	0.033	0.40[a]
1c	0.026	0.100	3.1	0.053	0.75
3b	0.162	0.260	327	0.140	>10
3c	0.015	0.085	64	0.050	6.7
3r	0.0022	0.093	8.9	0.090	1.1
3v	0.0028	0.245	11	0.027	0.38

[a] Tested po as the corresponding lactone.

Since suppression of extrahepatic cholesterol biosynthesis would not be expected to contribute significantly to plasma cholesterol lowering and may lead to undesirable consequences, several phosphorus-based compounds were evaluated as inhibitors of cholesterol synthesis from [^{14}C]acetate in both hepatic and nonhepatic cells (Table IV). Results obtained with lovastatin sodium (**1b**) and pravastatin (**1c**) are shown for comparison. The most striking difference between pravastatin (**1c**) and lovastatin sodium (**1b**) is the high degree of hepatocyte selectivity observed in the case of pravastatin. Pravastatin is a 31-fold weaker inhibitor of cholesterol synthesis in human skin fibroblasts[17] than in isolated rat hepatocytes[18] while lovastatin is a 7.7-fold more potent inhibitor in fibroblasts than in hepatocytes. Both the phosphonic (**3b**) and phosphinic (**3c**, **3r**, and **3v**) acid-based inhibitors show remarkably high levels of hepatocyte selectivity, being 50–1200-fold weaker inhibitors of cholesterol synthesis in fibroblasts than in hepatocytes. The upper limit of hepatocyte selectivity in this series is unprecedented with the known HMG-CoA reductase inhibitors.[19]

Table IV also summarizes the ability of the same compound to inhibit de novo cholesterol synthesis from [^{14}C]acetate on intravenous (iv) and oral (po) administration in a rat model.[20] Phosphinic acids **3c**, **3r**, and **3v**

(14) Compounds were assayed against rat microsomal reductase with 100 μM *R,S*-HMG-CoA and 2.7 mM NADPH. For a detailed description of this assay, see: Edwards, P. A.; Lemongello, D.; Fogelmann, A. M. *J. Lipid Res.* **1979**, *20*, 40.

(15) Indole-based dihydroxyheptanoic acid HMGR inhibitors have been reported by workers at Sandoz: Kathawala, F. G. PCT Int. Appl., WO 84/02131 A1, 1984.

(16) Indene-based dihydroxyheptanoic acid HMGR inhibitors have been reported by workers at Sandoz: Kathawala, F. G.; Wattanasin, S. PCT Int. Appl., WO 86/03488 A1, 1986.

(17) Faust, J. R.; Goldstein, J. L.; Brown, M. S. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 5018.

(18) (a) Berry, M. N. *Methods Enzymol.* **1974**, *32*, 625. (b) Capuzzi, D. M.; Margolis, S. *Lipids* **1971**, *6*, 601.

(19) Workers at Bristol-Myers have recently reported a dihydroxyheptenoic acid based HMGR inhibitor with a high degree of tissue selectivity (see ref 4e).

are about as effective as pravastatin and lovastatin in inhibiting cholesterol biosynthesis on iv administration in this assay. Indole (**3r**) and indene (**3v**) analogues also show oral activity equivalent to that of pravastatin and lovastatin in this assay and are also effective as hypocholesterolemic agents on chronic oral dosing in rabbits, dogs, and cynomologus monkeys.[21] On the basis of its overall pharmacological profile, the disodium salt of **3r** (SQ 33,600) has been chosen for clinical study in humans.

In summary, a new class of hydroxyphosphinyl-containing inhibitors of HMG-CoA reductase has been designed on the basis of mechanistic considerations of the enzymatic reaction. These compounds are as effective in inhibiting cholesterol biosynthesis in vitro and in vivo as compounds currently in clinical study. Several analogues appear to be at least 1 order of magnitude more hepatocyte selective than pravastatin, as estimated from their ability to inhibit cholesterol biosynthesis in fibroblasts and hepatocytes. The extension of this inhibitor design concept to other aromatic and heteroaromatic systems will be the subject of future disclosures.

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(20) In this assay male Sprague-Dawley rats adapted to a reverse light cycle were dosed with drug po 2.5 h or iv 2 h, 5 min before the middark cycle of maximum cholesterol biosynthesis. [^{14}C]Acetate was administered ip 2 h before middark and blood was drawn 2 h after middark. Plasma was separated and saponified and the nonsaponifiable lipids were counted. Percent inhibition of cholesterol biosynthesis was calculated from the percent decrease in the number of counts in the treated animals relative to controls. Plots of percent inhibition vs log dose were used to determine ED_{50}'s for the test compounds.

(21) Tanaka, R.; Arbeeny, C., unpublished results.

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Selective Inhibition of Urokinase by Substituted Phenylguanidines: Quantitative Structure-Activity Relationship Analyses

Altered proteinase regulation has been related to various pathological manifestations of altered cell function such as inflammatory disease, emphysema, and the development of invasive malignant behavior in tumors. Both synthetic and natural inhibitors of proteolytic enzymes have been widely studied.[1] High levels of expression by tumor cells of plasminogen activators, particularly urokinase (UK), have been implicated in cancer of digestive tracts, breast cancer, endometrial and cervical cancer, the metastasis of colon cancer, and mouse melanoma.[2] Autoimmune disease of the skin has also been reported to involve the excessive activity of UK.[3] Inhibitors of UK might be therapeutic agents for the above conditions or at least be used experimentally to assess the role of UK. Nevertheless, reports on reversible synthetic UK inhibitors have been few.

Geratz and co-workers[4] have investigated the inhibition of UK by a series of amidine compounds, among which the tightest binding to UK was displayed by bis(5-amidino-2-benzimidazolyl)methane with K_i of 2.3 μM. However, it also inhibited plasmin, trypsin, and thrombin with high potency, giving K_i values of 2.6 μM, 17 nM, and 4.15 μM, respectively.[4] In 1987 Vassalli and Belin[5] reported that amiloride, a potassium sparing diuretic drug, was an inhibitor of UK with K_i of 7 μM with selectivity over other serine proteinases including plasmin, tissue-type plasminogen activator (tPA), thrombin, and kallikrein. However, we found that amiloride is also a potent inhibitor of bovine trypsin with an apparent K_i of 17.2 μM at pH 8.3 when pyroGlu-Gly-Arg-*p*-nitroanilide (S-2444, Abbott Chemical and Agricultural Division, North Chicago, IL) was used as the chromogenic substrate. Therefore it was considered desirable to explore the possibility of developing small non-peptide compounds with selective inhibitory activity for UK over other serine proteinases.

UK hydrolyzes its biological substrate, plasminogen, at the arginyl-valyl bond 560 amino acids from the N-terminus. Although the structure of the active site of UK has not been experimentally elucidated, from the sequence homology and from comparative modeling studies[6,7] it is expected to have a binding pocket for a positively charged group as do the trypsin-like members of the serine proteinase family. After an initial screening of various ammonium, amidinium, or guanidinium compounds having aromatic or aliphatic side chains, we found that a series of simple phenylguanidine compounds are competitive inhibitors of UK with K_i values in the micromolar range (Table I).[8,11] The compounds were further tested on other

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(8) 4-Guanidinocinnamic acid hydrochloride and 4-(trifluoromethyl)phenylguanidine were synthesized from the corresponding amine by reacting with cyanamide under reflux.[9] 2-Aminophenylguanidine nitrate was synthesized by catalytic hydrogenation of 2-nitrophenylguanidine nitrate. 4-Guanidinobenzoic acid methyl ester hydrochloride was prepared from the acid by esterification in methanol and acetyl chloride.[10] The other phenylguanidine derivatives were purchased from Parish Chemical Co., Orem, UT. For the synthesized compounds, satisfactory NMR and elemental analyses were obtained.

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