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Perspective

2002 Alfred Burger Award Address in Medicinal Chemistry. Natural Products and Design: Interrelated Approaches in Drug Discovery

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The Alfred Burger Award in Medicinal Chemistry is named for a scientist whose contributions and leadership place him prominently in the history of medicinal chemistry in the United States. I am honored to join the distinguished group of chemists who have received this award and am grateful to the GlaxoSmithKline Company for sponsoring it.

My group's contributions in the discovery of the cholesterol-lowering statins and of ACE inhibitors for hypertension and more recently our use of privileged structures in the design of G-protein coupled receptor (GPCR) agonists are the subjects of this review. Each of these areas of research have been separately reviewed. In this historical overview, the focus will be on design issues and on the key role of natural products in providing proof of concept rationales and structural leads from which drug candidates and design ideas were evolved.

Discovery of Lovastatin

Our efforts in natural product discovery began in 1974 with the initiation of a fermentation products for screening (FERPS) project to supply microbial extracts for both in vitro and in vivo assays. Fungal and *Streptomyces* cultures were selected for use in the project primarily because they had already shown some microbial or other in vitro activity. Mechanistic homologies were assumed. For example, narrow-spectrum antibiotic activity might be produced by a peptidase inhibitor whose structure could be perfected subsequently for a mammalian peptidase target.

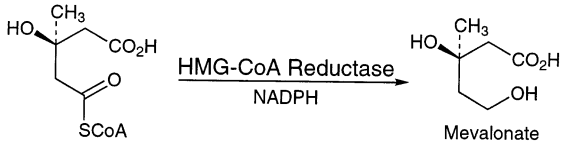
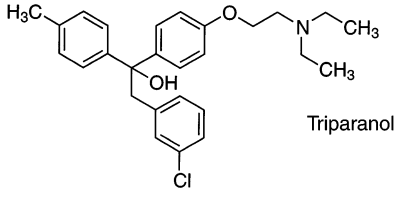


Figure 1. HMG-CoA reductase, the rate-limiting enzyme in the biosynthesis of cholesterol, generates mevalonic acid.

The first major success of the FERPS project came in 1978 with the isolation of lovastatin (then called mevinolin).[1] Evidence had been steadily growing in the 1970s that elevated plasma cholesterol levels correlate with an increased risk of coronary heart disease. To modulate its biosynthesis, a Merck group led by Albert Alberts decided to search for inhibitors of HMG-CoA reductase (Figure 1).[2] This is the rate-limiting enzyme in cholesterol biosynthesis, and the substrate that accumulates from its inhibition can go back into the metabolic pool.

At the time, an approach such as this was called "rational drug design" and was contrasted with animal assays with their low capacity and attendant safety concerns when specific mechanisms of action are not known. For example, the cholesterol lowering drug triparanol, whose discovery was made in animal models, unfortunately had to be withdrawn from clinical use.



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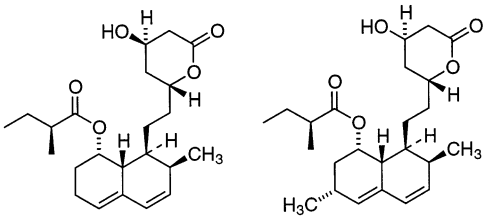


Figure 2. Structures of compactin (ML-236B, mevastatin) (**1**) and lovastatin (mevinolin) (**2**).

In patients, triparanol led to cataracts ascribed to a buildup of the late-stage intermediate desmosterol.[3,4]

Akiro Endo and associates of the Sankyo laboratories reported the first potent HMG-CoA reductase inhibitor in 1976.[5] This natural product, which they isolated from *Penicillium citrinum* and designated ML-236B, was also reported that year by A. G. Brown et al. of the Beecham Pharmaceutical laboratories as an antifungal agent produced by *Penicillium brevicompactum* (Figure 2).[6] Subsequently Endo and his associates reported that ML-236B (later called mevastatin) lowered cholesterol levels in animals and in man.[7−10]

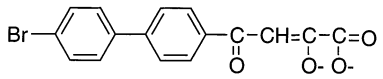
The *Asperigillus terreus* culture that produced lovastatin in the Merck laboratories was initially detected in an assay searching for folic acid biosynthesis inhibitors. This activity was not confirmed, but fungi were of interest to Richard Monaghan and he included this culture in the FERPS program. Its potent inhibition of HMG-CoA reductase was discovered in the laboratory of Albert Alberts, and his assay guided the isolation of lovastatin by Carl Hoffman and associates. Carl had isolated and synthesized mevalonic acid more than 20 years earlier with Karl Folkers and associates.[11−13] He had thus played a key role in discovering both the product of HMG-CoA reductase and a clinically important inhibitor of it. Endo also reported the isolation of lovastatin, which he called monacolin K.[14]

The cholesterol lowering properties in animals of lovastatin, whose K_i is 2- to 3-fold lower than ML-236B, have been described.[15] It increases LDL receptor numbers on hepatocytes, and this activity is primarily responsible for lowered LDL plasma cholesterol levels.

Clinical studies with lovastatin began in April 1980 but were interrupted in the fall of 1980 when the Merck Research Laboratories learned that the clinical testing of ML-236B had been stopped. An unconfirmed safety assessment problem with ML-236B was a serious concern since lovastatin and ML-236B differ by only one methyl group. However, the rationale for cholesterol lowering had become so compelling that lovastatin's use in patients with severe hypercholesterolemia was resumed in the summer of 1982 with the urgent support of several leading clinicians. Additional safety studies and extensive clinical testing culminated in the FDA approval of lovastatin in the late summer of 1987.[16] Subsequently additional statins have been introduced including totally synthetic variants. The use of statins in reducing the risk of cardiovascular diseases is now well established.[17]

Nakamura and Abeles analyzed the inhibitory properties of compactin and characterized it and, by inference, the other statins as bifunctional inhibitors.[18a] All of them contain the product analogue *des*-methylmevalonic acid linked to a hydrophobic anchor, which in the case of the natural product-based inhibitors is a perhydrodecalin derivative. Both components were estimated by Nakamura and Abeles to bind to HMG-CoA reductase with millimolar K_i values, yet when joined, the K_i of compactin in its active acid form is subnanomolar. Jencks had pointed out that huge gains in potency can be realized by simultaneous interactions on two sites of an enzyme.[18b] This strategy is mirrored today in methods such as "SAR by NMR" in which small molecular units are linked together to generate potent and specific ligands.[19]

Using a hydrophobic anchor to position and raise the potency of inhibitory functionality is an important design strategy. Nakamura and Abeles cite several examples.[18a] One from Merck is compound **3**, which is



3

an inhibitor of glycolic acid oxidase.[20] It is an end-product inhibitor ($IC_{50} = 6 \times 10^{-8}$ M) in which a bioisostere of the product of the enzymatic reaction, oxalic acid ($IC_{50} = 4.4 \times 10^{-4}$ M),[21] is linked to a hydrophobic biphenyl unit. In addition, conceptualizing GPCR privileged structures as hydrophobic anchors has been a basis for GPCR agonist designs, which will be described later.

ACE Inhibitors

The development of angiotensin converting enzyme (ACE) inhibitors was also greatly influenced by natural products. In this instance, the natural products were peptides from the venom of the Brazilian pit viper *Bothrops jararaca*. Originally they were isolated by Sergio Ferreira as bradykinin potentiating peptides.[22] Subsequently their activity as angiotensin converting enzyme inhibitors was demonstrated in the laboratories of J. R. Vane.[23−25] A Squibb group led by Cushman and Ondetti became interested in this research, and they also isolated and characterized active peptides from the snake venom.[26] In all, nine were characterized,[27] the most active of which in vivo as an ACE inhibitor was a nonapeptide designated teprotide (**4**) by the Squibb group. The most active in vitro was the pentapeptide BPP5a (**5**) (Figure 3).

<Glu-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro

4

<Glu-Lys-Trp-Ala-Pro

5

Figure 3. Snake venom peptide inhibitors of angiotensin converting enzyme: teprotide (BPP_{9a}, SQ20,881) (**4**) and BPP_{5a} (SQ20,475) (**5**).

Angiotensin II (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe) has potent hypertensive properties, and its biosynthesis involving renin and ACE was known (Figure 4). However, in the early 1970s it was not certain that the renin angiotensin system (RAS) was a good target, since elevated RAS activity had only been observed in a

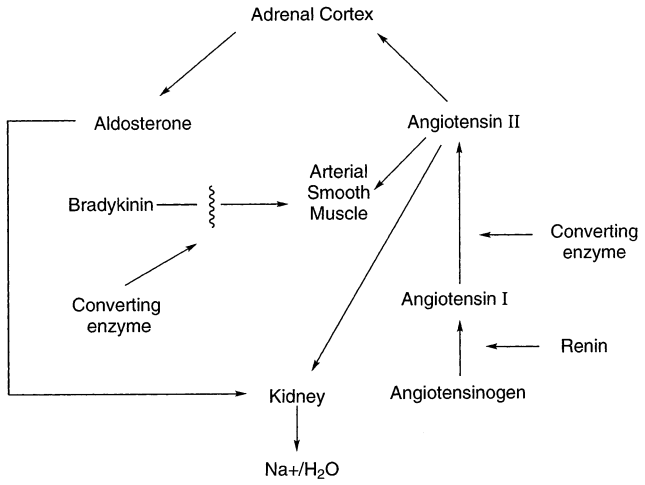


Figure 4. Schematic diagram of the renin−angiotensin system illustrating the multiple effects of angiotensin II in elevating blood pressure. Angiotensin converting enzyme generates angiotensin II by removing a C-terminal dipeptide from its inactive precursor angiotensin I (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu). Bradykinin, which has hypotensive properties, is also a substrate of angiotensin converting enzyme.

minority of patients with essential hypertension.[28] Nor was ACE necessarily the best target in the RAS, since its inhibition might result in side effects from elevated bradykinin levels or yet to be described substrates of the enzyme. Also, possibly the effects of ACE inhibition would diminish over time by compensatory increases in components of the RAS. Other targets might be preferable: renin, the rate-limiting enzyme in AII biosynthesis, or the AII receptor itself. These concerns were set aside with the publication of papers describing the clinical properties of teprotide that included efficacy in patients with normal RAS activity and the potentiation of ACE inhibition effects by diuretics.[29,30] Nor were there any limiting toxicities. Teprotide had to be given by injection, and it therefore had no potential as a major drug. However, its role in establishing a clinically important mechanism of action was decisive. It was widely realized that an orally active ACE inhibitor would be a valuable contribution to medicine. The challenge was how to synthesize one. Perhaps oral activity could be built into the snake venom peptides or a non-peptide lead found by screening.

The first solution to this problem was Ondetti and Cushman's design of captopril.[31,32] With brilliant insight they visualized BPP5a (**5**) binding to ACE as shown schematically in Figure 5. Then by positioning a thiol group for high affinity binding to zinc, BPP5a could be simplified to an orally active acylproline derivative. The result was captopril, the first marketed, orally active ACE inhibitor and an outstanding example of rational drug design.

Our synthetic program began shortly after the teprotide clinical results were published and focused primarily on phosphonates modeled after phosphoramidon, which is a natural product inhibitor of the zinc endopeptidase thermolysin.[36] However, good oral activity was not obtained. With the announcement of captopril, we abandoned phosphonates but continued to search for potent designs lacking the thiol group. The clinical side effects of captopril especially at high dose levels were

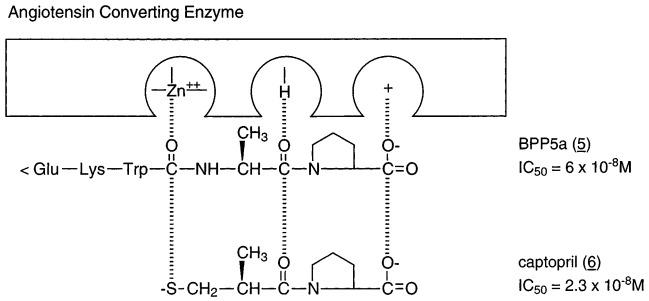


Figure 5. Angiotensin converting enzyme. The proposed binding of BPP_{5a} (**5**) and captopril (**6**) to the angiotensin converting enzyme. Adapted from Ondetti et al.[31]

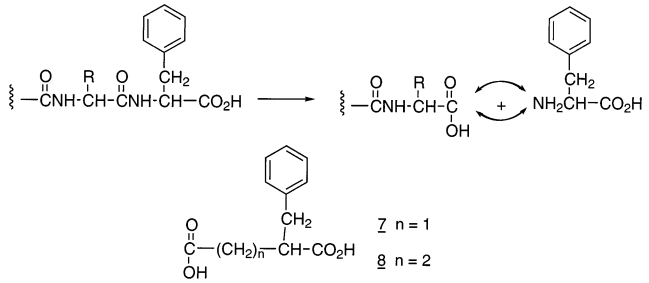


Figure 6. Byproduct inhibitors of the zinc metalloenzyme carboxypeptidase A designed by Byers and Wolfenden.[38,39]

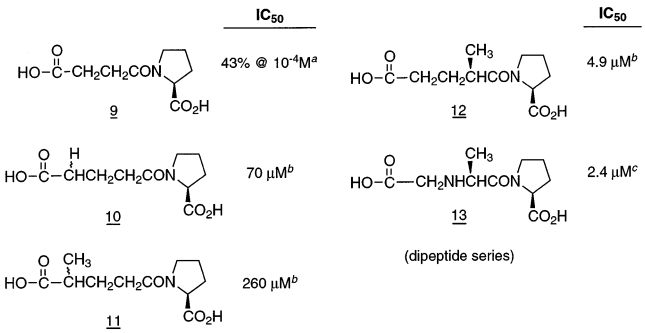
reported to include rashes, loss of taste, and proteinuria. We hypothesized the thiol group might contribute to them, since such side effects are also known with the thiol-containing chelator penicillamine, which Merck markets for Wilson's disease. Also, captopril is given orally 2−3 times a day and replacing the thiol group might increase metabolic stability.[37]

Efforts to achieve high potency with several non-thiol zinc ligands were unavailing, but a breakthrough came by adapting to ACE Wolfenden's "byproduct" design for the Zn^{2+} enzyme carboxypeptidase A (Figure 6).[38,39] Byers and Wolfenden reported L-benzylsuccinate (**7**) inhibited this enzyme with $K_i = 4.5 \times 10^{-7}$ M, and it was more effective than D,L-benzylglutarate (**8**) ($K_i = 5 \times 10^{-6}$ M). Their design concept was to gain potency by connecting important functionality of the weak binding products of an enzymatic reaction. Alan Maycock in our group tried this idea by synthesizing succinylproline when our program first began in 1974, but the approach was dropped when its low potency (43% inhibition at 10^{-4} M) was determined.[40]

The Squibb group also investigated the "byproduct" approach, and in one of their initial papers announcing captopril, they reported glutaryl, not succinyl, derivatives of proline had better potential as inhibitors of ACE. They also substituted each position of the glutaryl group with methyls, which enhanced activity only in **12** (Table 1).[32]

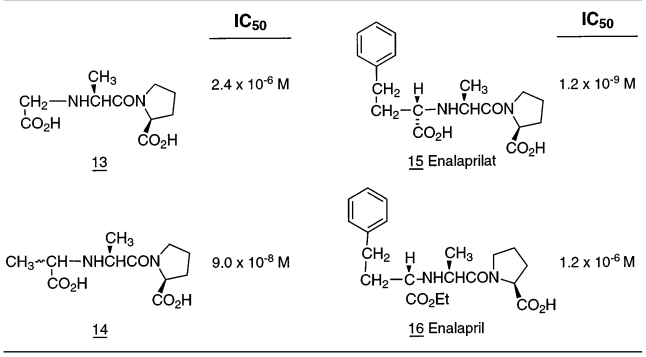
We recognized that nitrogen substitution in **12** would create a substituted Ala Pro unit and thus complete the "byproduct" design. Unfortunately this addition in **13** did not significantly increase activity, and the design was set aside for several months.[41] Counterbalancing factors were possibly responsible for an ineffectual result. For example, a favorable hydrogen bond might be offset by increased polarity in promoting solvated dissociation from the enzyme. The potency breakthrough came by adding a methyl group α- to the

Table 1. "Byproduct" Inhibitors of Angiotensin Converting Enzyme



[a] Maycock, A. L.; Patchett. Unpublished results, 1974. [b] Reference 32. [c] Reference 37.

Table 2. Enalapril and *N*-Carboxyalkyldipeptide Inhibitors of ACE[a]

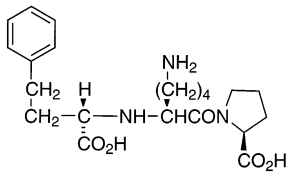


[a] Reference 37.

N-terminal carboxyl. The IC_{50} of this compound **14** as a mixture of two diastereomers was only 4-fold less than that of captopril and larger alkyls in this position markedly enhanced potency (Table 2).[37] As noted earlier, the added methyl group in the glutaryl analogue **11** decreased activity but apparently in compound **14** a hydrogen bond from the NH group helps position the adjacent methyl group toward the S_1 subsite of the enzyme. After extensive modifications were made in that position, the resolved phenethyl derivative enalaprilat (**15**) ($IC_{50} = 1.2 \times 10^{-9}$ M) was chosen for detailed evaluations. The addition of the phenethyl group as a hydrophobic anchor had raised the potency of **13** more than a 1000-fold.

Not surprisingly, the oral activity of enalaprilat was poor.[42] To correct this problem, syntheses of nonpeptides were continued and prodrug esters were made of enalaprilat. Facile diketopiperazine formation obviated esterification of the proline carboxyl. However, monoesters of the *N*-carboxyalkyl group afforded good oral activity, which was not importantly dependent on lipophilicity, so the ethyl ester enalapril (**16**) was selected for development. It is only weakly active as an ACE inhibitor ($IC_{50} = 1.2 \times 10^{-6}$ M)[37] and is deesterified to the potent ACE inhibitor enalaprilat (**15**) primarily in the liver.[42]

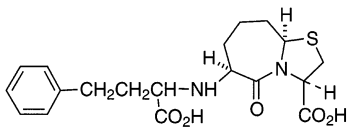
Active transport by a peptide carrier contributes to the oral absorption of enalapril.[43,44] Even more striking was the unexpected oral activity of lisinopril (**17**).[41] This excellent ACE inhibitor ($IC_{50} = 1.2 \times 10^{-9}$ M) was made in the course of systematic amino acid variation at each position in enalaprilat. It is a dizwitterion whose esterification would seem to have been required for oral activity given the enalaprilat experience. However, prodrug modification was not helpful and evidence for transport on a peptide carrier was subsequently published.[45]



17 lisinopril

Enalapril was approved for marketing by the FDA in December 1985 and lisinopril in December 1987. Both are widely used to treat hypertension and congestive heart failure and are available alone and in combination with hydrochlorothiazide.

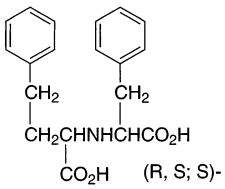
Non-peptide ACE inhibitors were reported from the Merck laboratories.[46,47] The most active of them is **18** with $IC_{50} = 6 \times 10^{-10}$ M.[48] It is severalfold more potent



18

than enalaprilat, although a dramatic conformational entropy advantage is not evident. Presumably it and enalaprilat have similar conformations in solution and on the enzyme.

Extension of the *N*-carboxyalkylpeptide design to other Zn^{2+} metallopeptidases has been summarized,[49] but it is noteworthy the design as exemplified by **19** did not work well for carboxypeptidase A ($K_i = 5 \times 10^{-5}$ M).[50] If this fact had been known at the onset of the



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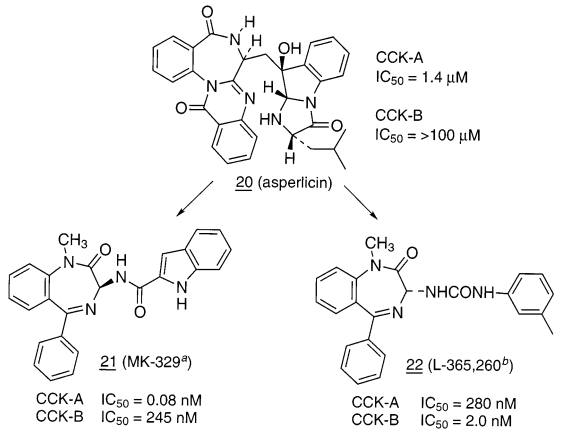
program, the *N*-carboxyalkyl design might never have been tried to generate ACE inhibitors such as enalaprilat.

Asperlicin and Privileged Structures

Isolation of the cholecystokinin A antagonist asperlicin (**20**) from *Aspergillus alliaceus* was the second major achievement of the fermentation products for screening project. This natural product is a competitive antagonist of CCK-33 in rat pancreatic tissue ($IC_{50} = 1.4\ \mu$M), and it blocked CCK-8 induced guinea pig gall bladder contractions when given intravenously at 12 mpk.[51]

Asperlicin itself did not have sufficient potency or oral activity to be a drug candidate. To correct these deficiencies, a lead development program was undertaken by chemists at the Merck laboratories at West Point, PA. In a remarkable strategy, they simplified design

Table 3. Potent Cholecystokinin CCK-A and CCK-B Antagonists Generated from Asperlicin (**20**)



[a] Reference 52. [b] Reference 53.

considerations to the benzodiazepine core of asperlicin and from it they were able to elaborate potent, orally active analogues selective for both the CCK-A and CCK-B receptors (Table 3).[52,53] Subsequently in a similar way Yu et al. developed a class of CCK-B antagonists by derivatizing the quinazolinone core of asperlicin.[54]

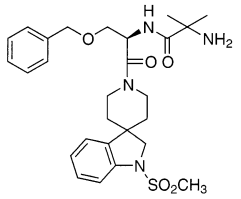
The strategy of elaborating leads from core scaffolds is beautifully illustrated in these CCK antagonists. Evans and co-workers called scaffolds such as benzodiazepines "privileged structures".[55] Furthermore, "what is clear is that certain 'privileged structures' are capable of providing useful ligands for more than one receptor and that judicious modification of such structures could be a viable alternative in the search for new receptor agonists and antagonists".[55] This influential observation, made in 1988, came at a time when combinatorial chemistry was entering a period of rapid growth. The selection and derivatization of scaffolds that recur in classes of bioactive compounds became a strategy to complement diversity in the generation of combinatorial chemistry libraries for lead discovery.

At Merck, our focus was on structural scaffolds in agonists and antagonists of G-protein coupled receptors. Our contribution was to derivatize them with capped amino acids and dipeptides. Importantly these peptidyl privileged structures have afforded small-molecule agonists of much larger natural ligands and excellent receptor selectivities have been achieved.[56]

The first success using this approach was MK-0677 (**25**),[57] which was developed as a functional mimetic of the hexapeptide growth hormone secretagogue GHRP-6 (**24**).[58] Subsequently ghrelin (**23**), the natural ligand of the growth hormone secretagogue (GHS1a) receptor, was discovered.[59] MK-0677 and ghrelin bind to the GHS1a receptor with comparable affinity, and both are full agonists of this receptor. However, MK-0677 is a capped dipeptide and ghrelin is a 28 amino acid peptide bearing an octanoyl group on Ser^3 (Table 4).

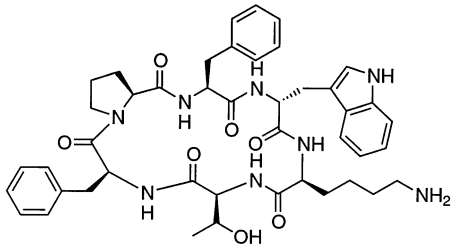
A reverse turn structure based on spectroscopic, molecular dynamics, and analogue studies has been proposed for GHRP-6.[60] MK-0677 and GHRP-6 are competitive ligands, and their functional activities both require Glu124 in TM3 of this receptor.[61] These data support a proposed superposition of MK-0677 on the turn structure of GHRP-6.[62] Similar modeling against

Table 4. Growth Hormone Secretagogues

	IC_{50}[a]
Gly-Ser-OctSer-Phe-Leu-Ser-Pro-Glu-His-Gln-Arg-Val-Gln-Gln-Arg-Lys-Glu-Ser-Lys-Lys-Pro-Pro-Pro-Ala-Lys-Leu-Gln-Pro-Arg 23 ghrelin	0.71 ± 0.6 nM
His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2 24 (GHRP-6)	13.2 ± 0.13 nM
 25 MK-0677	0.51 ± 0.2 nM

[a] Determined by the displacement of [^{125}I]ghrelin from cloned human GHS1a receptor expressed in COS-7 cells. Unpublished data of Sharon Sadowski of the Merck Research Laboratories, Rahway, NJ.

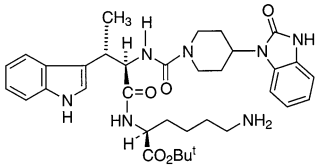
the key β-turn functionality of somatostatin hexapeptide agonists such as compound **26**[63] led to the development



26

of dipeptidyl privileged somatostatin agonists including **27** (Table 5).[64] Unlike somatostatin, which binds well to all of its receptor subtypes, **27** binds selectively to SSTR2 and it does so as a full agonist with affinity equal to the much larger somatostatin-14.

Table 5. Binding Affinities of Somatostatin-14 and Compound **27** to Somatostatin Receptor Subtypes[a]

		Ki (nM)
Ala–Gly–Cys–Lys–Asn–Phe–Phe–Trp Cys–Ser–Thr–Phe–Thr–Lys Somatostatin-14	hsst1	0.38
	hsst2	0.04
	hsst3	0.66
	hsst4	1.76
	hsst5	2.32
 27	hsst1	2,392
	hsst2	0.01
	hsst3	31
	hsst4	81
	hsst5	163

[a] Reference 64.

Finally the peptidyl privileged structure design has led to potent and selective melanocortin MC4R agonists.[65] Thus, the formulation of privileged structure strategies[55,56] from work on asperlicin initiated an extensive and continuing chain of research activities in our laboratories.

Current Trends

Natural product screening today is largely focused on antibiotics, immunosuppresants, and cancer therapy. Fermentation and plant products have done well in these areas of research, and molecular biology and genomics are providing many new targets. In metabolic diseases the natural product ligands are often peptides. Improved efficiencies in the design of small-molecule agonists and antagonists will make important contributions to this research. Many of them will likely not be peptidomimetic as strictly defined but will have allosteric mechanisms as our understanding of structure and function increases of enzymes, receptors, ion channel, and transport proteins.

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Biography

Dr. Arthur A. Patchett is a retired Vice President of Medicinal Chemistry at Merck Research Laboratories, Rahway, NJ. He is a graduate of Princeton University, was a Fulbright Scholar at Cambridge University, and was awarded a Ph.D. in organic chemistry from Harvard in 1955. He spent 2 years at the National Institutes of Health before joining Merck in 1957. He retired in 2000 but remained as a Merck consultant for 2 years. Dr. Patchett is the coauthor of 172 publications and 182 U.S. patents. He was Chairman of the American Chemical Society Division of Medicinal Chemistry in 1971. His honors include the Pharmaceutical Manufacturers Association Discoverer's Award, the E. B. Hershberg Award, and the Smissman Bristol-Myers Squibb Award. He twice received the Directors' Scientific Award of Merck & Co.

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