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E. B. Hershberg Award Address

Excursions In Drug Discovery

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I am highly honored to be the 1993 recipient of the E. B. Hershberg Award for Important Discoveries in Medicinally Active Substances sponsored by the Schering-Plough Corp. Dr. Hershberg was an outstanding research director. During his tenure as Director of Research, the Schering group produced drugs of great value to medicine including the anti-inflammatory steroid prednisolone and the aminoglycoside antibiotics gentamycin and neomycin. His scientific leadership was admired throughout the industry.

I am being honored primarily for my contributions in the design of the antihypertensive drugs enalapril and lisinopril and in the discovery of the cholesterol-lowering drug lovastatin. I will recount that work in some detail but, in addition, I would like to reflect upon broader aspects of my career. I have titled this talk "Excursions in Drug Discovery" because my associates and I over the years have attempted to contribute to many different areas of medicinal chemistry with approaches that variously involved natural products, lead modifications, and de novo design. We never became specialists. Looking back over 38 years, my career seems to have been a series of explorations in search of new directions rather than exhaustive studies of any subject.

My training in organic chemistry owes much to Professor Robert B. Woodward who directed my Ph.D. research, culminating in the synthesis of lanostenol. I was in awe of his intellectual prowess and his commitment to elegance and style in the conduct of research. Medicinal chemistry is a more complex, empirical science than is organic chemistry, but there are opportunities to make elegant drug designs and seizing them, whenever possible, in my opinion, is one of our best ways of making lasting contributions.

After Harvard, I did not know if I wished to follow a career in academics or in the pharmaceutical industry. So, I decided to do research at the National Institutes of Health for several years to expose myself to biomedical research. It was an enriching experience and I have been caught up in the importance of drug discovery ever since. My mentor was Dr. Bernhard Witkop. He taught me to appreciate amino acid and peptide research and introduced me to the challenges involved in unraveling biorganic mechanisms. His scholarship and historical perspective set a tone in his research that, to this day, reminds me to see our own efforts in the context of the giants in research whose achievements we are trying to extend.

I came to Merck in 1957 to work with Dr. Lewis H. Sarett. My years of apprenticeship in drug discovery continued with him until 1972. I joined Merck because of its reputation in research and, in particular, I was greatly impressed by Sarett's total synthesis of cortisone. Important anti-inflammatory steroid research was going on and one of my early assignments was to analyze the structure–activity relationships of these compounds.[1] That task taught me one of the fundamental principles of medicinal chemistry. Even making small changes, such as adding unsaturation or fluorine or methyl groups, can have profound effects on potency and, in this case, on the sodium-retention properties of corticosteroids, even though these molecules had presumably been perfected by evolution over millions of years. We had only rudimentary ideas of receptors, but in analyzing steroid structure–activity relationships, it was evident that quite specific dimensions must distinguish the receptors responsible for anti-inflammatory and sodium-retention responses. Unfortunately, until our knowledge of ligand interactions with receptors becomes more precise, one never knows what parts of a ligand should be modified to enhance receptor selectivity. Sometimes profound alterations in one part of a molecule yield no therapeutic advantage. The phenylpyrazolocorticosteroids, which were designed by

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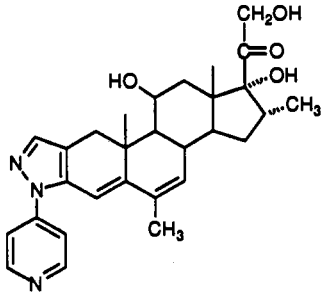
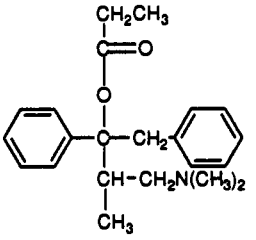
Hirschmann and co-workers,[2] illustrate this point. Later, Dr. John Hannah and I designed some very potent topical anti-inflammatory steroids using this approach.[3] The best of these, compound 1, was active clinically in the treatment



(1)

of psoriasis, but it had no advantages in comparison with other topically active steroids of the day and, thus, it was not developed.

Our efforts in the analgesic field taught me that making drugs is not easy. Many unexpected events can and do intervene between concept and the *in vivo* realization of one's goals. Our task was to improve upon the low addiction potential of propoxyphene (2) by restricting the



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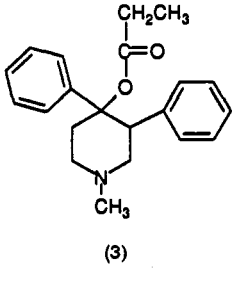
conformations of this molecule in the hope that binding would still occur to the analgesic receptor and not to one hypothetically associated with addiction. These efforts led to compounds 3[4] and 4[5] (MK-137). DL-α- and



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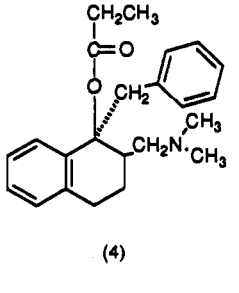
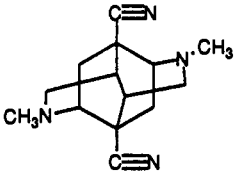


(4)

-β-isomers of the former were inactive as analgesics. Presumably, their restricted conformations did not allow binding to the analgesic receptor; however, in accord with good receptor fit, compound 4 was more potent than propoxyphene. Nonetheless, the onset of tolerance with 4 was remarkably and unexpectedly fast. For this reason, its planned development was stopped. We still like to restrict molecules to their bioactive conformation hoping receptor specificity will be obtained, but we learned early that this is only one of several strategies which can be tried and may fail for one or more of a host of possible reasons.

Other contributions in this formative phase of my career were in the total synthesis of 18,19-bisnorsteroids bearing phenyl[6] and amino[7] groups at the 18-position and an aromatic C-ring analog of 18-norestrone.[8] These compounds were to be intermediates for 19-norsteroidal birth control pills, but Merck management decided to withdraw from the fertility-control field before their chemistry was further developed. My group synthesized guanidino penicillins and cephalosporins[9] and the fascioliacide rafoxanide[10] which was marketed for some years as Ranide. We also prepared analogs of the growth promotant zearalenone[11,12] and of the antibiotic fosfomycin[13] which is used clinically overseas in combination with aminoglycoside antibiotics.

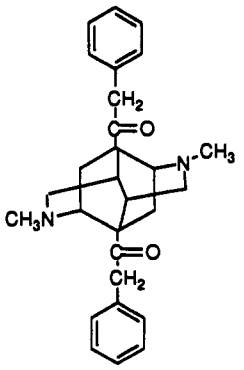
The research activities which I have mentioned were guided by Dr. Lewis H. Sarett. His leadership derived from a brilliant, analytical mind and an intense desire to innovate both in science and in research strategies. In the late 1960s and early 1970s, he became concerned that progress in new drug discovery was slowing down. To address the problem, one of his management strategies was to create a department called New Lead Discovery. Its mission was to augment testing with new types of compounds represented poorly or not at all in our sample collection. Dr. Ralph Hirschmann led the department for a year before I was asked to assume responsibility in 1972. Early strategies placed emphasis on the random generation of compounds, which as a concept we see again today in the synthesis of combinatorial compound libraries. For example, our peptide collection was built up, and photochemically driven dimerization and rearrangement reactions were explored. We also synthesized core molecules which we could derivatize in various random ways. In my opinion, the department's best achievements using this strategy were the diazaditwistanes derived from the core structure 5 synthesized by Grabowski and co-



(5)

workers.[14] It was possible to make hundreds of molecules from 5 by mono or bis variations at the cyano and N-positions which were acids, esters, amides, Grignard products, alkylation products, etc. It is an interesting molecule. The cyano-derived substituents are in the β-position of one piperidine ring and in the γ-position of another. Biological activities might be anticipated since piperidine rings are one of the most common, recurring templates in medicinal chemistry. Most interestingly, the diazaditwistanes have an axis of symmetry. Thus, the cyano positions are identical even in respect to absolute stereochemistry, although the molecule as a whole is resolvable. We were intrigued by the possibility of designing dual-acting drugs using a diazaditwistane core, but a good opportunity to do this did not arise.

Our most interesting discovery was compound 6, which as the (−)-isomer was approximately 3 times as potent as



(6)

morphine in the rat.[15] In retrospect, based on its structure, I suspect 6 may have κ-opioid properties although we have never gone back to determine this.

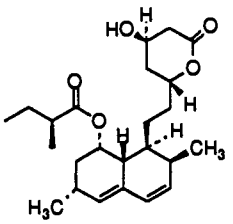
By the mid 1970s, one of the most far-reaching changes in drug discovery was underway. No longer were the biologists trying to design more and more elegant animal models to simulate human disease. Instead, biochemical targets had become the focus: enzymes, receptors, and ion channels whose inhibition might moderate a disease process or eliminate a microbe, virus, or parasitic organism with minimal effect on the host. When completely successful, the resultant drugs should produce only mechanism-related side effects. And today, one can be quite confident that inhibitors of cloned human receptors will be active in man if good pharmacokinetic properties are realized.

The implications of mechanism-oriented discovery are enormous. Only small quantities of compound are required for initial testing and actives can be found in mixtures of compounds which would be toxic in whole animals. Furthermore, improvements in HPLC, NMR, and mass spectrometry in the 1970s were making the identification of compounds in complex mixtures a more easily achievable task.

To take advantage of this situation, we organized in 1974 the Fermentation Products for Screening Project (FERPS) in which broth extracts were sent for biochemical assays while these assays were being developed for high-volume testing. This was a time of great enthusiasm for natural product screening. Microbial sources provide a rich diversity of molecules which had largely been untried in pharmacology since one cannot inject whole broth extracts into animals and expect to find much. Since we could only turn out 20 extracts per week, selection strategies were needed. One of them was to use prescreens in which the initial target was, if possible, an entire biosynthetic pathway. For example, we were interested in folic acid biosynthesis inhibitors for possible utility as antibacterial or antiprotozoal agents. In short, we were looking for a naturally occurring sulfa drug. To screen for such compounds, Dr. Richard W. Burg of the Merck Microbiology Department developed an assay based on reversal of antimicrobial activity by a cocktail of folic acid dependent metabolites. This assay was run in high-volume screening for Merck in the CEPA (Compania Espanola de Penicilina y Antibioticos) Laboratories in Madrid and cultures showing promise were sent back to Rahway for regrowth and further testing. The activity of one of them, an *Aspergillus terreus*, was not confirmed in the folic acid assay. Nonetheless, Dr. Richard L. Monaghan, who directed the microbiology aspects of FERPS, felt it was an interesting culture and, since this was also part of our selection process, he had it regrown for broad testing.

So, an extract of the *A. terreus* culture was prepared in the FERPS laboratory by Dr. John Rothrock and Ms. Maria Lopez and sent off for the various biochemistry assays testing FERPS at the time. These assays included one for HMG-CoA reductase, the rate-controlling enzyme in the synthesis of cholesterol. The search for inhibitors of this enzyme had been initiated several months earlier by Mr. Alfred W. Alberts. He and our research director, Dr. P. Roy Vagelos, continuing their lifelong interest in lipid metabolism, had become convinced that cholesterol's role in cardiovascular disease was established and that its control should be a prime target of rational drug discovery. In November 1978, Ms. Julie Chen in Mr. Alberts' laboratory put this *Aspergillus terreus* extract into the HMG-CoA reductase assay, and its remarkable activity was discovered. We had not had much success with FERPS testing until then and were beginning to wonder if microorganisms can only produce antibiotics and the occasional antiparasitic or antitumor drug. Thus, with great anticipation and skill, the FERPS team headed by Dr. Carl H. Hoffman set about isolating the HMG-CoA reductase inhibitor as rapidly as possible. A good producing culture and a reliable assay helped immensely. Within weeks, a small sample of pure lovastatin (then called mevinolin) was obtained. By remarkable coincidence, Dr. Hoffman had isolated mevalonic acid 22 years earlier with Dr. Karl Folkers. Since mevalonic acid is the product of the HMG-CoA reductase enzyme, Dr. Hoffman had played a key role both in the isolation and control of this key building block in cholesterol biosynthesis. There is indeed serendipity in research as I hope this story of the lovastatin (Mevacor) discovery illustrates. The next step was structure determination, which was achieved with remarkable speed by Dr. Georg Albers-Schonberg and his associates in our Department of Biophysics.

An account of the lovastatin (7) discovery was published in 1980.[16] During the first 6 months following its isolation,



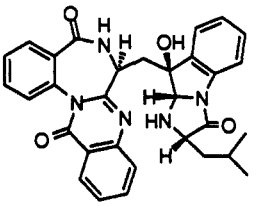
(7)

the FERPS group crystallized it, prepared adequate quantities for *in vivo* and initial toxicity studies, and isolated a dihydro analog.[17] We also synthesized reduction and cyclopropanation products.[18] Responsibility for additional analogs was undertaken with great success by Dr. R. L. Smith and his associates at the Merck Research Laboratories (MRL) in West Point, PA, and the cholesterol-lowering drug Zocor resulted from those efforts.

The isolation of lovastatin had come 4 years after the start of the FERPS program and after testing nearly 5000 fermentation extracts in numerous enzyme and receptor assays. Lovastatin, through the efforts of many individuals, was approved for use in the United States in 1987. After more than 5 years of clinical use, it is now established

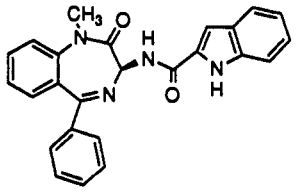
as safe, front-line therapy to reduce the risk of hypercholesterolemia in causing coronary heart disease.

The other major discovery to come out of the FERPS project was asperlicin (8).[19] It is only a modest antagonist



(8)

of the cholecystokinin A receptor (IC_{50} = 1.4 μM) and its oral activity was poor. Nonetheless, asperlicin reinforced our conviction that microorganisms elaborate natural products able to interact with mammalian receptors. In the case of asperlicin, analogs of a most imaginative kind were required to raise potency, obtain oral activity, and antagonize selectively both the peripheral and brain CCK receptors. One might have supposed the need for more elaborate structures to obtain higher potency. Instead, brilliant insights by Dr. Ben Evans, Dr. Mark Bock, and their associates in the Merck laboratories in West Point, PA, led them to focus upon and modify only the benzodiazepine core of asperlicin. Compound 9 [MK-329; IC_{50}



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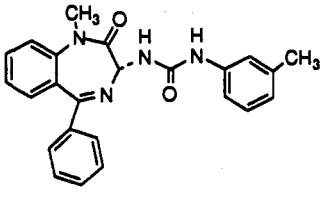
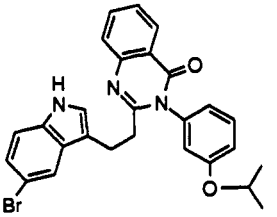


(10)

(CCK-A) = 0.08 nM][20] and compound 10 [L-365,260; IC_{50} (CCK-B) = 2.0 nM][21] are outstanding achievements from their approach. Quite strikingly, researchers in the Lilly laboratories were able to simplify asperlicin in a different way. They focused upon its quinazolinone core and elaborated that structure to a CCK-B-selective antagonist, compound 11 (IC_{50} = 81 nM).[22] Thus, the natural product

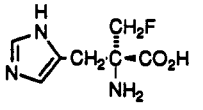


(11)

asperlicin exemplifies the concept of two overlapping receptor antagonist pharmacophores in a single molecule. We often see more than one structural type affording antagonists of the same receptor. Here, two structural types are present in the same molecule.

Returning to the chemical objectives of the New Lead Discovery Department, it had become evident by the mid 1970s that the random synthesis of compounds for screening was not a cost-effective way of making new discoveries. In its place, we decided to organize an enzyme inhibitor design project and enlisted two expert consultants, Drs. Richard Wolfenden and Robert Abeles, to advise us in transition state and suicide inhibitor design strategies. Later, Drs. Christopher Walsh, Jeremy Knowles, and Dan Rich assisted our enzyme inhibitor projects. We achieved two of our goals: the design of potent, *in vivo* active inhibitors of pyridoxal-dependent enzymes, and we synthesized potent inhibitors of metallopeptidases. Primarily, I wish to comment on Zn^{2+} peptidases and the development of angiotensin converting enzyme inhibitors, but before doing so, I would like to describe briefly our work with pyridoxal-dependent enzymes.

We became interested in designing suicide inhibitors of the latter enzymes as a consequence of Dr. Janos Kollonitsch's synthesis of fluoro-D-alanine,[23] the basis of whose antibacterial activity is alanine racemase inhibition. Its mechanism of action was established to involve an anionic intermediate, elimination of fluorine, and enzyme alkylation.[24] We extended the design to amino acid decarboxylases because their mechanism also involves a pyridoxal Schiff base stabilized anion. Specific, irreversible inhibitors of glutamic acid, ornithine, dihydroxyphenylalanine (DOPA), and histidine were synthesized.[25] Of these, the histidine analog 12 generated the greatest

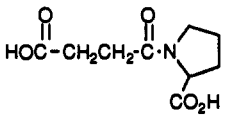


(12)

interest in our laboratories. It reduces histamine levels most rapidly in tissues in which there is a high turnover of histamine and it is active, especially when chronically administered, in various animal models of allergic inflammation, ulcers, and asthma. (Fluoromethyl)histidine was studied extensively, including Phase I clinical testing, but ultimately Merck management decided not to develop it since effective and selective histamine H_1 and H_2 antagonists could be drawn upon for most of the clinical conditions that can be treated with (fluoromethyl)-histidine.

Returning to metallopeptidases, interest at Merck in the one of them, the angiotensin converting enzyme (ACE) was intensified in 1974. A landmark paper appeared then describing blood pressure lowering in a small group of hypertensive patients treated with intravenously administered teprotide. Importantly, this peptidal ACE inhibitor was effective in some patients whose renin levels were normal and no side effects were reported.[26] Although limited in scope, this study raised the possibility that ACE inhibitors could be broadly useful in controlling hypertension. Teprotide pointed the way, but oral activity was needed.

A screening assay for ACE inhibitors was set up by Dr. Edward H. Ulm in late 1974 and we began to send compounds to him for testing. Also, Dr. Alan Maycock joined the New Lead Discovery Department from Dr. Abeles' laboratory in 1975. One of his first tasks was to extend the Byers and Wolfenden biproduct design[27,28] for the metalloenzyme carboxypeptidase A to the design of ACE inhibitors. Structure–activity studies with the snake venom peptides had been published,[29] and thus, the importance of a carboxy-terminal proline in peptide inhibitors was recognized. An *N*-succinoyl derivative of proline (compound 13) was synthesized by Dr. Maycock since benzylsuccinic acid is more active than benzylglutaric



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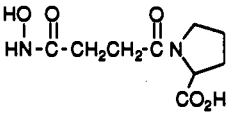
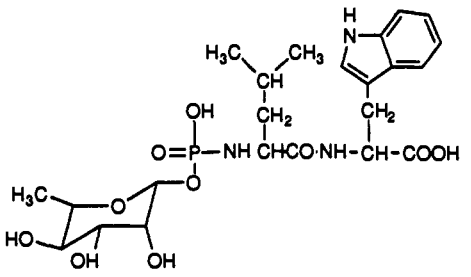
acid as an inhibitor of carboxypeptidase A. Furthermore, since ACE was known to be a Zn^{2+} enzyme, the hydroxamic acid (14) was also prepared. Much to our disappointment,



(14)

the potencies of these compounds were poor: 43% inhibition at 3.3×10^{-4} M for compound 13 and $IC_{50} = 50$ μM for compound 14.[30] We turned away from biproduct designs and decided to synthesize ACE inhibitors based upon phosphoramidon (15) which is a potent natural



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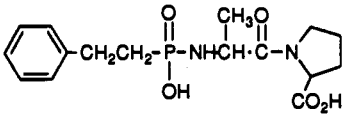
product inhibitor of the metallopeptidase thermolysin. It was possible to synthesize highly active inhibitors using this approach, for example, compound 16 ($IC_{50} = 7$ nM),[31] but we were not able to obtain oral activity.



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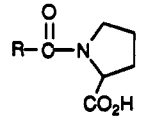
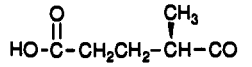
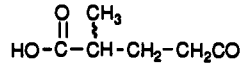
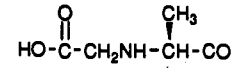
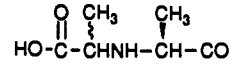
The Squibb group led by Drs. Ondetti and Cushman announced their elegant design of the orally active ACE inhibitor captopril in 1977.[32,33] Early reports on captopril indicated usage three times a day, and some side effects were experienced, including rash and loss of taste. We increased our manpower at that point, believing that a compound with improved duration of action and less side effects might be realized if the sulfhydryl group of captopril could be eliminated. However, direct substitutions of imidazoles, sulfonamides, iminosulfonamides, tetrazoles, and catechols as ligands to Zn^{2+} did not afford useful levels of inhibition.[34] The problem was 2-fold: how to eliminate the SH group but retain both potency and oral bioavailability.

In tracing the evolution of captopril from (carboxyalkanoyl)prolines, the Squibb group reported a result that was surprising to us: α-methylglutaroylproline (17, $IC_{50} = 4.9$ μM; Table I) was more potent than the α-methylsuccinoyl analog. Most importantly, this finding presented an opportunity to perfect a biproduct design since inserting an NH group into the α-methylglutaroyl group provides

Table I. Biproduct Derivatives of Proline

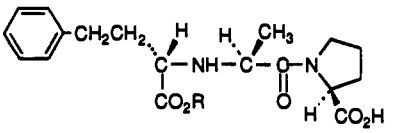


compd	R—C	IC_{50}, μM
17		4.9[a]
18		260[a]
19		2.4[b]
20		0.09[b]

[a] Reference 33. [b] Reference 36.

a fully expressed AlaPro unit in compound 19. Dr. E. E. Harris synthesized this compound but, much to our dismay, with an $IC_{50} = 2.4$ μM, it was no more potent within experimental error than was reported for the (α-methylglutaroyl)proline prototype. We set aside this design for 3 months but decided to come back to it, feeling that equivalent activity despite major physical differences (arising from a potentially protonated NH in place of a CH_2) suggested counterbalancing effects. Perhaps the basicity of the NH group should be reduced or compound 19 should be made more hydrophobic. Dr. Matthew Wyvratt took the latter approach and added the second methyl group in compound 20. Its activity ($IC_{50} = 0.09$ μM), even as an *RS*-compound, was close to that of captopril and the breakthrough that led to enalapril and lisinopril had been made. Fortunately, we had forgotten in the Squibb paper compound 18 ($IC_{50} = 260$ μM), whose poor potency might have suggested that substitution α to the terminal carboxyl group would be poorly tolerated. Evidently, the NH group helps to position the added methyl group in compound 20 into the S_1 subsite of the enzyme with a resultant major increase in potency.

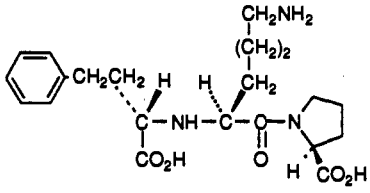
With a breakthrough in hand in September 1978, all of the synthetic chemists in the department joined in making analogs of compound 20, and enalaprilat (21, $IC_{50} = 1.2$



R = H (21) (enalaprilat)
Et (22) (enalapril, *Vasotec*®)

nM) was soon synthesized. The properly positioned phenethyl group in enalaprilat had increased the activity of compound 19 by 1000-fold! Enalaprilat was not well-absorbed orally, but we were fortunate that a simple ethyl ester derivative, enalapril (Vasotec, compound 22) corrected that deficit. Enalapril is a prodrug which has negligible ACE inhibitory activity until it is de-esterified *in vivo*. To obviate the possibility of patient-to-patient variability in the activation of a prodrug, our next goal was to synthesize an orally active compound which did not require metabolic activation. Dr. M. T. Wu and his

associates achieved this goal unexpectedly in the course of studying alanine replacements in enalaprilat. Soon after the initial potency breakthrough, the focus of the project was not on intrinsic activity but on achieving optimal oral activity and duration of action. Directing these critical *in vivo* evaluations were Drs. Charles S. Sweet and Dennis M. Gross. It was they who discovered the excellent *in vivo* properties of the lysine analog of enalaprilat. The oral activity of this potent but polar compound **23**



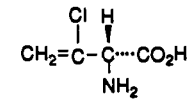
(23) (lisinopril, *Prinivil®, Zestril®*)

(lisinopril, IC_{50} = 1.2 nM) was not predicted by the chemists. After the event, we suspected that peptide transport might be involved and, thus, we synthesized some lysine analogs into whose butyl chain we introduced amide groups and heteroatoms to enhance similarity with peptides. None of those changes seemed to increase oral activity further. However, quite recently evidence has been presented that peptide transport is involved in the oral absorption of lisinopril.[35]

Announcement of the discoveries of enalapril and lisinopril were made in 1980.[36] Enalapril entered intensive clinical testing in that year and its use in the treatment of hypertension received NDA approval in December 1985 after testing in over 3000 patients in eight Phase III multicenter trials. After more than 6 years of use, it is an established therapy for hypertension and congestive heart failure. Lisinopril has also earned a significant place in medicine as Prinivil and Zestril. I cannot begin to credit the many individuals who contributed to the development of enalapril and lisinopril. However, I would like to mention Dr. Ralph F. Hirschmann, who was my mentor during this and the lovastatin projects. Constant focus on the interface between biology and chemistry is one of the cornerstones of his success in research and as a manager. It is at this interface that drugs are discovered. Thus, Hirschmann's emphasis on structural biology and on the productive interactions of chemists and biologists greatly supported these achievements.

The *N*-(carboxyalkyl) part-structure of enalapril is present in most of the ACE inhibitors now used in therapy. We applied it also in the design of thermolysin[37] and neutral endopeptidase 24.11 inhibitors,[38] and the design is now widely used to inhibit metallopeptidases.[39]

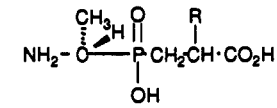
Our research interests following upon the ACE inhibitors have included the synthesis of inhibitors of bacterial cell wall enzymes. Progress in the discovery of new antibiotics from microbial sources had slowed in the 1980s. We were convinced that chemists could design inhibitors of some of the unique enzymes which are critical to bacterial growth and chose as our targets alanine racemase and alanine ligase. The background for these choices was the broad spectrum antibacterial combination MK-641/MK-642, in which the former is the racemase inhibitor [2-^{2}H]-3-fluoro-D-alanine and the latter is a prodrug of the alanine ligase inhibitor cycloserine. We were already quite familiar with fluoro-D-alanine and its mechanism of inhibition of the pyridoxal-dependent enzyme alanine racemase as discussed earlier. To make a better inhibitor of this enzyme, our goal was a compound which would not produce the toxic metabolite fluoropyruvate, nor would the compound be recognized as a D-alanine equivalent and be incorporated in the cell wall in its place. These criteria were met with the synthesis of D-(chlorovinyl)glycine (**24**), which is a time-



(24)

dependent, irreversible inhibitor of alanine, whose efficiency as judged by its partition ratio was superior to fluoro-D-alanine.[40,41]

The synthesis of potent alanine ligase inhibitors was achieved with phosphinic acids such as compound **25**.[42] It

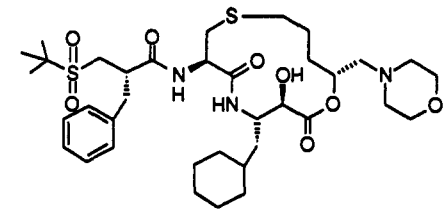


R = (RS)-heptyl (25)
(R)-methyl (26)

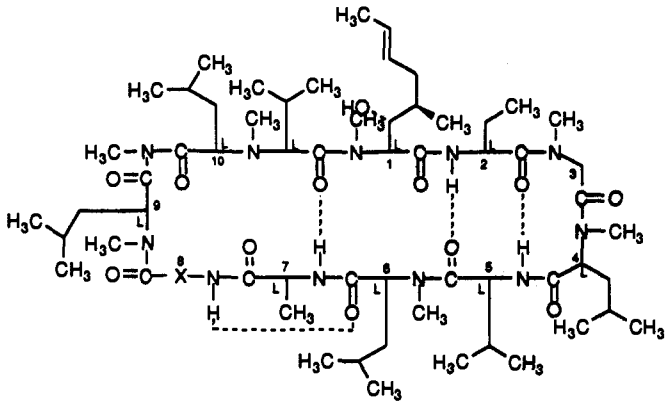
was found that ATP enhances their binding to the enzyme[43] and Dr. Walsh and co-workers established that phosphorylation of the phosphinic acid group is responsible for their extremely tight binding.[44] In fact, the $t_{1/2}$ to regain enzyme activity is 17 days with inhibitor **26**.[45]

Unfortunately, neither the (halovinyl)glycines nor the amino alkylphosphinates are good antibacterial agents, presumably because they penetrate bacteria poorly. Limited attempts to improve antibacterial spectrum by making peptide conjugates of them were not successful. No doubt, with better understanding of bacterial transport processes, the prospects will be brighter to design effective antibacterial agents *de novo* using these and other bacterial enzyme targets. However, a recent spin-off of the work arose from the realization that fluoro-D-alanine would be a good precursor for the biosynthesis of modified cyclosporins. The latter contain D-alanine in the 8-position and, when fluoro-D-alanine is added to the producing culture, production of D-alanine is halted and fluoro-D-alanine enters the cyclosporin molecule in its place.[46] Subsequent treatment with a large excess of an aprotic base yields a dehydroalanine intermediate, to which thiol compounds can be added to form 8-substituted cyclosporins, such as compound **27**.[47] Several of these new cyclosporins are active immunosuppressants and they remain of interest.

Our group has recently published on potent macrocyclic renin inhibitors[48,49] including compound **28**, whose IC_{50} is



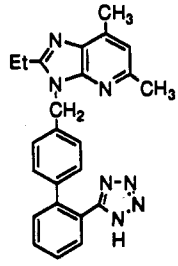
(28)



(27)

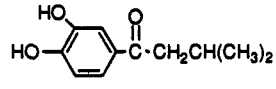
X = CHCH2SCH3 (D-configuration)

0.35 nM (human renin). We have also announced AII antagonists such as compound **29** (L-158,809), which shows



(29)

excellent oral activity and an IC_{50} = 0.3 nM on the angiotensin AT_1 receptor.[50] Our involvement in the latter project has made us increasingly interested in ways to accelerate receptor antagonist discovery and in learning how receptor agonists and antagonists function at the molecular level. As an initial step in the latter direction, we assisted Dr. Catherine Strader in the discovery of compounds that are able to activate a mutant β-adrenergic receptor. She inactivated that receptor by removing the key Asp^{113} amine-binding residue from it and substituting a Ser^{113} in its place. We then set about finding compounds that would fully activate the wild-type receptor. Compound **30** will do just that, although its potency is only



(30)

micromolar and it is yet to be optimized.[51] The mutagenesis experiments of Dr. Strader's group established the importance of Asp^{113} in this receptor when the ligand is an amine, such as isoproterenol. The fact that a nonbasic agonist for the mutant receptor could be found that does not activate the wild-type receptor is consistent with a functionally important role for residue 113. It is interesting that ketone **30** is a full agonist of the Ser^{113} mutant βAR, suggesting that interaction with the residue at position 113 is a key determinant of agonist affinity but that other regions of the receptor are important for efficacy. These findings also raise the possibility of developing compounds specifically effective in transgenic animals which bear a genetically engineered receptor. Perhaps someday, for example, compounds will be synthesized which have physiologic properties, such as growth promotion only in transgenic food animals bearing a mutant receptor and be devoid of effects on the human wild-type receptor if ingested by people.

Looking toward the future, I believe there are many opportunities for progress in drug design. We are learning how to enhance chemical screening with the aid of computer systems and how to develop leads for structurally defined targets using molecular modeling and X-ray crystallography. We must also acquire greater knowledge of transport and metabolism processes. Current major stumbling blocks in drug development are often the clumsy, empirical, and time-consuming efforts required to go from an exquisitely potent *in vitro* inhibitor to one with good bioavailability and an adequate duration of action. This is the unglamorous part of drug development, but it often separates highly successful ventures from those which lag behind them. We need to become more efficient in our strategies to improve the *in vivo* properties of drugs and we must keep perfecting our understanding of structure and toxicity relationships. Improvements in these areas will contribute immensely to the efficiency with which rationally defined biochemical targets can be translated into important drugs.

In closing, I acknowledge with many thanks the Schering-Plough Corp. for sponsoring this award and my many associates who participated in my excursions in drug discovery. Their ideas and hard work made the journey a very stimulating one.

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