CHAPTER ONE

Reflections on Medicinal Chemistry at Merck, West Point

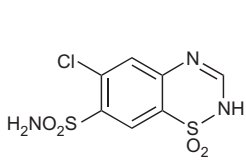
Paul S. Anderson
Lansdale, PA, USA

Much has changed since I arrived at Merck's West Point laboratories in 1964 as an organic chemist interested in learning how to do drug discovery. It quickly became apparent that this endeavor would require a prolonged period of study and learning. Later, I realized that it would, in fact, require life-long learning. The learning experience began immediately as others taught me the process by which therapeutic targets were selected and pursued at that time. Medicinal chemistry was make a one compound at a time endeavor that mainly relied on data from experiments in whole animals for SAR information. Safety pharmacology and drug metabolism studies were reserved mostly for development candidates. Despite these limitations, the pharmaceutical industry had been remarkably successful in the discovery of new medicines. One such example was the thiazide diuretics.

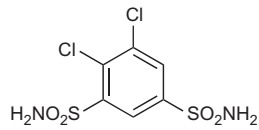
The West Point laboratories had successfully discovered and developed the thiazide diuretic chlorothiazide **1**.[1] At the time, whole animal pharmacology was central to this major accomplishment. The biological assays in place tended to measure pharmacological activity without necessarily defining the mechanism by which the activity was achieved. It was clear, however, that physiological understanding of kidney function had played a critical role in the course of events that led to the discovery of the thiazide diuretics. The discovery road began with an earlier observation that the antibacterial agent sulfanilamide produced alkaline diuresis in patients. This effect was tracked to inhibition of carbonic anhydrase in the kidney. Carbonic anhydrase inhibitors induced excretion of sodium bicarbonate by the kidney. Inhibition of sodium reabsorption by exchange of sodium for hydrogen ion in the distal part of the kidney therefore was thought to account for the alkaline diuresis induced by these enzyme inhibitors. Karl Beyer set forth the idea that a drug that worked in the proximal portion of the kidney would be better tolerated by excreting sodium chloride instead of sodium bicarbonate.[2] His efforts to find such a molecule in collaboration with medicinal chemists James Sprague and Frederick Novello led to the

Annual Reports in Medicinal Chemistry, Volume 47
ISSN 0065-7743
http://dx.doi.org/10.1016/B978-0-12-396492-2.00001-1

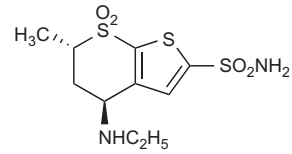
discovery of dichlorfenamide **2**, a potent carbonic anhydrase inhibitor with increased chloride secretion relative to acetazolamide. Further SAR work revealed that addition of an amino group to the aromatic ring of bis-sulfonamides decreased carbonic anhydrase inhibition without loss of chloride secretion. A subsequent molecule in which the amino group and a sulphonamido group were incorporated into a ring became known as chlorothiazide **1**, a potent diuretic that did not inhibit carbonic anhydrase. It was the first of many thiazide diuretics that found broad clinical use in treating edema and hypertension.



1



2



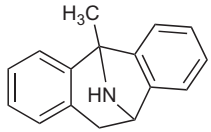
3

Interestingly, carbonic anhydrase reappeared as a target of interest at Merck in the 1980s as Robert Smith and his colleagues sought to design an inhibitor for topical use in the eye to treat glaucoma. Although many carbonic anhydrase inhibitors were known, those previously approved for clinical use as oral medications failed to lower intraocular pressure on topical administration. We believed that a topically administered drug would avoid the side effects associated with the use of the oral medications. Eventually, we were successful in finding such an inhibitor. Success required gaining knowledge about ocular transport issues from *in vitro* models and finding the right animal model for testing these insights *in vivo*. It also required the identification of a potent carbonic anhydrase inhibitor that was soluble in water and had good transport properties as well as a long residence time in the ciliary process tissue where the enzyme resides in the eye. At West Point, Jack Baldwin accomplished this difficult task with the discovery of dorzolamide **3**, the first topically effective carbonic anhydrase inhibitor for the treatment of glaucoma.[3] In the course of this work, we had learned that the challenges of getting acceptable physical, metabolic, and transport properties in a drug candidate could be even greater than those associated with optimization for potency and selectivity. One of the ancillary benefits of this program was an early opportunity to explore the use of X-ray crystal structures of an inhibitor bound to an enzyme in drug discovery.[4,5] This early experience in the manipulation of physical properties and the use of protein–ligand crystal structures in drug design would pay a dividend in later work on the design of inhibitors for HIV protease.

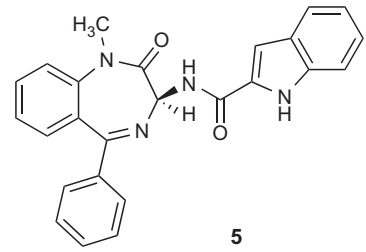
In the 1970s, Merck had initiated a program to expand access to new compounds being synthesized in academic chemistry departments by offering to screen these materials for biological activity under a collaborative agreement. One compound that proved to be interesting was 5,6,7,8-tetrafluoro-1,4-dihydronaphthalene-1,4-imine, which unexpectedly was found to have benzodiazepine-like behavioral activity as well as potent antiseizure activity in rodents without the usual sedative side effects of this well-known class of compounds. Pursuit of this interesting observation led to a more drug-like molecule dizocilpine **4**, better known as MK-801.[6] Studies focused on the mechanism of action of MK-801 used (^{3}H)MK-801 to identify specific high-affinity binding sites in rat brain membranes that proved to be associated with NMDA receptors. Neurophysiological studies were then used to establish that MK-801 was a noncompetitive antagonist of the NMDA receptor.[7] While MK-801 was not developed as a drug, it has served as a valuable, commonly used tool in neuropharmacology. The MK-801 work taught us a great deal about the importance of mechanism of drug action studies in drug discovery work.

While the MK-801 work was in progress, Dan Veber's group at West Point was pursuing several other opportunities for drug discovery based on hypotheses that antagonists of certain peptide hormones might be useful therapeutic agents. The peptide hormone CCK was selected as the initial target based on the current knowledge of its physiology and pharmacology. CCK-A receptors were believed to mediate pancreatic and biliary secretion as well as gut motility, while CCK-B receptors were implicated in gastric acid secretion and panic–anxiety attacks. The plan was to screen fermentation broths for molecules with affinity for CCK receptors using a radio receptor assay in the hope of finding nonpeptidal compounds that would prove to be antagonists. The screening effort identified asperlicin as a modestly potent nonpeptidal CCK antagonist. Subsequent fragmentation and reassembly analysis of the natural product sought to identify and optimize the pharmacophore responsible for this activity. As the project proceeded, Ben Evans and Mark Bock were successful in designing **5** and **6** that were orally bioavailable, potent, and selective antagonists for CCK-A and -B receptors, respectively.[8,9] While these molecules demonstrated the expected pharmacological consequences of selective receptor blockage, neither became a drug because of side effect and efficacy issues. A similar approach to the study of the peptide hormone oxytocin also gave rise to new nonpeptidal antagonists that were not subsequently developed as drugs. It became apparent that there are many targets for which a

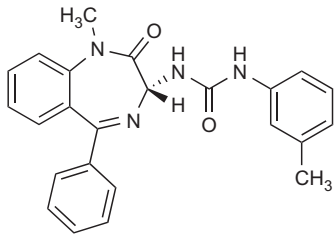
preclinical hypothesis for a mechanism of drug action can be developed, but few of these hypotheses actually translate to new medicines for a variety of reasons. One that did achieve the desired objective was the West Point effort to discover and develop an antagonist for the αIIbβ3 integrin receptor, which recognizes the RGD sequence present in many integrins. The Merck effort led by Ruth Nutt and George Hartman produced both peptide and nonpeptide antagonists. The nonpeptide antagonist was developed as tirofiban **7**,[10] which was approved by the FDA in 1998 for treatment of unstable angina. These experiences with MK-801 and the search for peptide antagonists as new medicines taught us that target selection is a critical component of the drug discovery process that requires careful analysis before committing to a course of action. A clear understanding of what will be required to translate the preclinical hypothesis into a successful clinical outcome needs to be a key part of this analysis.



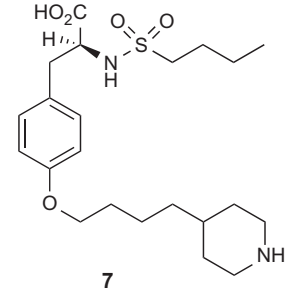
4



5



6

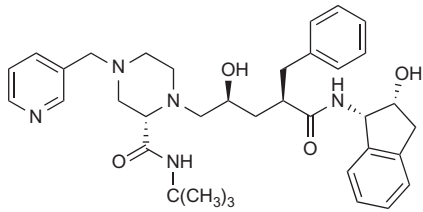


7

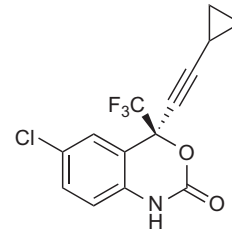
Discovery of drugs to manage HIV infection was a major goal of the pharmaceutical industry during the closing years of the twentieth century. Once human immunodeficiency virus was identified as the etiological agent of AIDS, understanding of molecular events in viral replication revealed the virus-specific enzymes HIV protease, reverse transcriptase, and integrase to

be excellent targets for therapeutic intervention. Both established and early stage pharmaceutical companies joined the effort to find drugs that worked by these mechanisms. At Merck, our entry into the fray was spearheaded by the synthesis of HIV protease as an initial source of the enzyme for inhibitor assay development and protein crystallographic studies.[11] Recognition of HIV protease as an aspartic acid protease provided insight for inhibitor design gleaned from earlier study on other aspartic acid proteases such as renin. The Merck sample collection contained a number of peptides and peptide-like molecules that had been designed to be renin inhibitors. These molecules all had a core secondary alcohol component believed to be a transition-state mimic for the cleavage site in substrates. Several of these renin inhibitors were found to also be HIV protease inhibitors. While they provided guidance for the SAR optimization work, they lacked potency in cells and were not orally bioavailable. X-ray crystal structures of HIV protease with and without early inhibitors bound to it facilitated the design process, as did molecular modeling. These structures confirmed the importance of proper positioning of the secondary alcohol in the enzyme active site and revealed other interesting interactions between inhibitors and the flap region of the active site. With these insights in hand, the march toward molecules with high potency in cell culture was fairly rapid, however, finding molecules with acceptable ADMET properties proved to be challenging. Very few molecules satisfied the ADMET criteria for development as an orally active drug. Eventually, the West Point team led by Joel Huff and Joe Vacca overcame these challenges with the discovery of indinavir **8**.[12]

Nonnucleoside reverse transcriptase inhibitors also have become important in the treatment of HIV infection. Screening of small molecule sample collections for leads to this class of compounds was an important step toward their discovery. Several companies including Johnson & Johnson, Boehringer-Ingelheim, Merck, and Pharmacia Upjohn obtained leads from their sample collections that evolved into clinical candidates. The Merck lead evolved into the widely used drug efavirenz **9**, which was discovered by Steve Young.[13] The Boehringer-Ingelheim lead progressed to nevirapine and the Pharmacia Upjohn compound became delavirdine. These inhibitors bind to a flexible, allosteric site in the enzyme, which may account for the structural diversity permitted by this target. Crystal structures of inhibitors bound to this site were subsequently obtained. Although this information did not contribute to the early work on nonnucleoside inhibitors, it has enhanced our understanding of this flexible binding site.



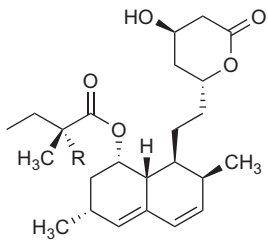
8



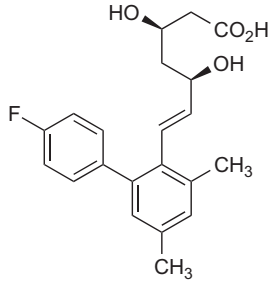
9

One of the most important contributions to medicine in the second half of the twentieth century was the development of the statins. The conversion of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) to mevalonic acid by the enzyme HMG-CoA reductase is the rate-limiting step in cholesterol biosynthesis. Thus this enzyme was an obvious target for the discovery of drugs with utility in controlling cholesterol in the blood. Subsequent understanding of the regulatory role that HMG-CoA reductase plays in LDL receptor expression further enhanced the desire to find inhibitors with drug-like properties. An enzyme assay suitable for use in screening fermentation broths for inhibitory activity was developed and used to discover natural product inhibitors such as compactin and lovastatin **10**. Merck developed and launched lovastatin as a product for controlling blood cholesterol level. This successful product served as a template for further work to improve its drug-like properties. It was noted that metabolic hydrolysis of the ester in lovastatin gave rise to a metabolite with much less enzyme inhibitory activity. Increasing bulk by adding a methyl group alpha to the ester carbonyl in lovastatin enhanced metabolic stability and more than doubled *in vivo* potency. The new molecule called simvastatin **11** became a very successful treatment for hypercholesterolemia.[14] Both of these inhibitors are prodrugs because the active species is the beta hydroxy acid formed on metabolic hydrolysis of the lactone. It was assumed that this part of the inhibitor resembled an intermediate in the reaction pathway of HMG-CoA reductase and therefore was key to how the enzyme recognizes and binds to this class of inhibitor. This assumption suggested that it might be possible to replace the stereochemically complex decalin part of simvastatin with a less complex hydrocarbon structure. Exploration of this hypothesis at Merck led to the discovery of a family of biphenyl replacements for the decalin substructure such as **12**.[15] A common feature of these and latter HMG-CoA reductase inhibitors was the *p*-fluorophenyl entity thought at the time to be a replacement for the hydrophobic ester present in simvastatin. Subsequent X-ray crystal structures of enzyme-bound inhibitors validated this concept.[16]

The new biphenyl inhibitors were very potent but did not appear to have additional preclinical advantages that merited clinical development. However, other companies pursued these structures to arrive at fluvastatin and atorvastatin using a strategy that often is referred to as variation of a known active compound. Over time, this has been one of the most productive approaches to the discovery of new medicines, as one can readily see by reviewing the discovery of beta blockers, histamine antagonists, ACE inhibitors, and many other majors classes of drugs.



10 R = H
11 R = CH_3



12

The above advances in health care offered useful teaching about the nature of drug discovery. Studying the physiology of organ function can put one on a productive track for drug discovery, as was the case with the thiazide diuretics. Understanding mechanism of drug action also has great value, as was the case with the integrin antagonists, statins, and HIV infection treatments. It was clear that the hurdles for drug discovery are high. Discovering a molecule that is selective for the desired mechanism of drug action and has an acceptable ADMET profile for the intended route of administration is a formidable challenge even with the tools that are available today. In most projects, very few molecules satisfy all of the necessary criteria.

New tools such as genomics, metabolomics, bioinformatics, high-throughput screening and chemistry, fragment-based drug design, and structure-based drug design have been incorporated into the drug discovery process without fundamentally changing the paradigm or increasing productivity. This can be put into perspective by realizing that technology supports drug discovery, but people discover drugs. Process makes you functional, but process does not make you creative. Creativity is still a key factor in the transformation of a drug discovery concept into a new medicine. To this end, target selection is key, because most new target opportunities begin

with a preclinical hypothesis that lacks formal clinical validation. History tells us that most of these hypotheses fail to achieve clinical validation. However, it is likely that some of the new tools may eventually reduce failure rate by helping drug discovery scientists to pick the best targets for the discovery of new medicines. To this end, Sir David Jack[17] has reminded us that successful organizations engage all of their people in the selection of the best ideas for drug discovery with recognition that these are likely to be the ones that are "simple, practicable with available resources and novel enough to yield medicines that are likely to be better than probable competitors in ways that will be obvious to both doctors and their patients." In a related statement, George Merck addressed the challenge of balancing business interests and the interest of patients by saying in a 1950 speech at the Medical College of Virginia, "We try to remember that medicine is for the patient. . .it is not for the profits. The profits follow, and if we have remembered that, they have never failed to appear. The better we have remembered it, the larger they have been." It would be good for us to remember this as the drug discovery enterprise moves forward in the twenty-first century. The pharmaceutical industry has undergone changes in recent years such that building shareholder value has come to dominate creation of value for patients and doctors. Better balance is needed between business interests and the interests of patients and their physicians in order to have a productive industry that can better serve the healthcare needs of society in the twenty-first century. A return to better balance will enable creative medicinal chemists to continue to discover important new medicines.

REFERENCES

(1) Novello, F.C.; Sprague, J.M. *J. Am. Chem. Soc.* **1957**, *79*, 2028.
(2) Beyer, K.H. *Trends Pharmacol. Sci.* **1980**, *1*, 114.
(3) Sugrue, M.F.; Harris, A.; Adamsons, I. *Drugs Today* **1997**, *33*, 283.
(4) Baldwin, J.J.; Ponticello, G.S.; Anderson, P.S.; Christy, M.E.; Murcko, M.A.; Randall, W.C.; Schwam, H.; Sugrue, M.F.; Springer, J.P.; Gautheron, P.; Grove, J.; Mallorga, P.; Viader, M.P.; McKeever, B.M.; Navia, M.A. *J. Med. Chem.* **1989**, *32*, 2510.
(5) Smith, G.M.; Alexander, R.S.; Christianson, D.V.; McKeever, B.M.; Ponticello, G.S.; Springer, J.P.; Randall, W.C.; Baldwin, J.J.; Habecker, C.N. *Protein Sci.* **1994**, *3*, 118.
(6) Thompson, W.J.; Anderson, P.S.; Britcher, S.F.; Lyle, T.A.; Thies, J.E.; Magill, C.A.; Varga, S.L.; Schwering, J.E.Z.; Lyle, P.A.; Christy, M.E.; Evans, B.E.Z.; Colton, C.D.; Holloway, M.K.; Springer, J.P.; Hirshfield, J.M.; Ball, R.G.; Amato, J.S.; Larsen, R.D.; Wong, E.H.F.; Kemp, J.A.; Tricklebank, M.D.; Singh, L.; Oles, R.; Priestly, T.; Marshall, G.M.; Knight, A.R.; Middlemiss, D.N.; Woodruff, G.N.; Iversen, L.L. *J. Med. Chem.* **1990**, *33*, 789.

(7) Wong, E.H.F.; Kemp, J.A.; Priestley, T.; Knight, A.R.; Woodruff, G.N.; Iversen, L.L. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 7104.
(8) Evans, B.E.; Bock, M.G.; Rittle, K.E.; DiPardo, R.M.; Whitter, W.L.; Veber, D.F.; Anderson, P.S.; Freidinger, R.M. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 4918.
(9) Bock, M.G.; DiParto, R.M.; Evans, B.E.; Rittle, K.E.; Whitter, W.L.; Veber, D.F.; Anderson, P.S.; Freidinger, R.M. *J. Med. Chem.* **1989**, *32*, 13.
(10) Lynch, J.F.; Cook, J.J.; Sitko, G.R.; Holahan, M.A.; Ramjit, D.R.; Mellott, M.J.; Stranieri, M.T.; Stabilito, I.I.; Zhang, G.; lynch, R.J.; Manno, P.D.; Cjang, C.T.-C.; Egbertson, M.S.; Halczenko, W.; Duggan, M.E.; Laswell, W.L.; Vassallo, L.M.; Shafer, J.A.; Anderson, P.S.; Friedman, P.A.; Hartman, G.D.; Gould, R.J. *JPET* **1995**, *272*, 20.
(11) Nutt, R.F.; Brady, S.F.; Darke, P.L.; Ciccarone, T.M.; Colton, C.D.; Nutt, E.M.; Rodkey, J.A.; Bennett, C.D.; Waxman, L.H.; Sigal, I.S.; Anderson, P.S.; Veber, D.F. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 7129.
(12) Vacca, J.P.; Dorsey, B.D.; Schleif, W.A.; Levin, R.B.; McDaniel, S.L.; Darke, P.L.; Zugay, J.; Quintero, J.C.; Blahy, O.M.; Roth, E.; Sardana, V.V.; Schlabach, A.J.; Graham, P.I.; Condra, J.H.; Gotlib, L.; Holloway, M.K.; Lin, J.; Chen, I.-W.; Vastag, K.; Ostovic, D.; Anderson, P.S.; Emini, E.A.; Huff, J.R. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 4096.
(13) Young, S.D.; Britcher, S.F.; Tran, L.O.; Payne, L.S.; Lumma, W.C.; Lyle, T.A.; Huff, J.R.; Anderson, P.S.; Olsen, D.B.; Carrol, S.S.; Pettibone, D.J.; O'Brien, J.A.; Ball, R.G.; Balani, S.K.; Lin, J.A.; Chen, I.-W.; Schleif, W.A.; Sardana, V.V.; Long, W.J.; Barnes, V.W. *Antimicrob. Agents Chemother.* **1995**, *39*, 2602.
(14) Hoffman, W.F.; Alberts, A.W.; Anderson, P.S.; Chen, J.S.; Smith, R.L.; Willard, A.K. *J. Med. Chem.* **1986**, *29*, 849.
(15) 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.
(16) Istvan, E.S.; Deisenhofer, J. *Science* **2001**, *292*, 1160.
(17) Jack, D. *Creating the Right Environment for Drug Discovery*. Quay Publishing: Lancaster, **1991**.