What Type of Structure-Activity Correlation Succeeds in Drug Design?*

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To discover new drugs, it helps to know what you want and why. Consequently, structure-activity relationships have become a rewarding tool. Experiences in using this tool are described, particularly as used by the chemist and biologist team.

When I entered the laboratories of the then Sharp and Dohme from the Department of Physiology at the University of Wisconsin Medical School many years ago, it was to gain chemical support for the kind of biology that interested me. To discover new therapeutic agents took more of everything in research than I have ever had. The biology I brought with me was serviceable, but the organic chemistry was not much better than a great respect for the impact of the physical aspects of organic structure on the behavior of biological reactions. Or maybe I should say, the impact of the physical aspects of organic reactions on biological structures. After these many years, I am not sure it matters which or whether both are right, for what I shall have to say is surely passé in terms of an announcement that came across my desk the other day on a course in drug design. The course dealt with things I surely hope our younger medicinal chemists know about, like how to do semi-empirical molecular orbital calculations; mathematical quantitative structure/activity models, linear free-energy related quantitative structure/activity relationships. I think it would be great fun to know such things and still be able to discover new drugs. My problem was always how to get around what I didn't know. By leaning on each other, James Sprague and I managed, and I'll tell you how with the aid of a few examples.

First let me tell how I decided not to be a chemist. The graduate students in physiology when I was at Wisconsin somehow all managed to get involved with various sympathomimetic amines. We begged compounds from one place or another until we had a lot of different structures and knew quite a bit about what they did to various bodily functions and what happened to them. Having done a fair amount of the work myself, especially on the metabolic side, it seemed to me that the really interesting compound would be one we didn't have. The things I wanted in the molecule were utility, activity, and stability. Intellectually, these attributes were built into the basic phenethylamine in the opposite order of stability, activity, and utility by alpha methyl substitution on the side chain and the election of the metahydroxy nucleus. I made the compound, and it worked fine. The side chain was stable to amine oxidase, and the ring was not further hydroxylated by phenol oxidase, which was the case for the *p*-analog. These were the primative enzyme systems available for such study at the time. Pressor potency and duration of action were excellent—cardiac rhythm wasn't upset at substantial pressor dosages, etc. I still have a smidgen of the now brown stuff. It had been made before, but making what would now be called *dl*-metaraminol or *dl*-Aramine was hard enough for me. When I went to Sharp and Dohme, a real chemist by the name of Edward Engelhardt resolved the isomers, and the compound 1-*m*-hydroxyphenylisopropanolamine was marketed. It turned out rather well, considering FDA hasn't taken the drug off the market yet. The clinician knows of Aramine in shock, and the pharmacologist of the current generation has rediscovered metaraminol as a false transmitter.

That first experience was rewarding, but it taught me that there was plenty of room for what both good chemists (which I wasn't) and biologists had to offer in this kind of research. It taught me that to discover useful drugs it helps to know what you want and why. The experience taught me a respect for structure/activity relationships that has been a rewarding faith.

Anyway, if I can't exactly tell you how to do it, I can give you a few examples of how we happened on a drug or two that you know. Remember now, this started back in the days when research didn't spend its time chasing down adverse reactions, doing bioavailability studies, and trying to distinguish between possibly effective and probably effective established products in the line.

As I have already said, it helps to know what you want to do. Not like let's cure cancer or let's block a critical step in a vital or ubiquitous enzymatic reaction just to lower blood pressure. Biology and useful drugs are more subtle than that. Way back when the rapidity of penicillin excretion was an enigma, there probably were many ways we could have elected to decrease its output—like finding a drug to reduce glomerular filtration rate; or trying to alter renal cortical blood flow, or trying to stabilize penicillin binding to albumin. All of these approaches could be made to sound profound in a grant application. Less profound, but more appealing to many chemists (and biologists) would have been to screen milligram quantities of a hundred compounds a day on the aseptic excretion of a measured amount of penicillin administered intraperitoneally to mice in sufficient numbers to permit statistical analysis of the urinary recovery data. We didn't. To make this part of the story short, we knew that some compounds were secreted by the renal tubules. Finding that penicillin did the same in dogs was one of the most thrilling experiences of a young lifetime.

The idea that we might competitively inhibit the tubular secretion of penicillin had precedence in fact and in concept, but the next step is the one that counts so far as the chemist is concerned. That step, expressed as a question, is "where do we start?" Well, phenol red was secreted by the renal tubules, so was Diodrast, *p*-aminohippurate, some sulfonamides, and still other compounds. Any of these might have served as a lead for the chemist. We chose *p*-aminohippurate.

The enzymologists knew about competitive inhibition. The renal physiologists knew about the renal tubular secretion of organic acids like *p*-aminohippuric. Since we found

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that penicillin was secreted by the renal tubules, it seemed reasonable that a competitive inhibitor of that process might be a useful agent. But why start with *p*-aminohippurate for the synthetic program? An alternative would be to start by pulling a lot of organic acids off the shelf for testing. We didn't. I remember thinking about this and talking with my chemist partner, James Sprague, about it. We decided to build on *p*-aminohippuric acid, which made both biologist and chemist happy in this instance. Hippuric acid was an old friend of the biologists. It was the first metabolite ever isolated, and its *p*-amino analog had been thoroughly examined by Homer Smith for the measurement of renal tubular functional capacity. *p*-Aminohippurate had plenty of places for the chemist to work with, and it wasn't too far from the sulfonamide chemistry our chemists knew well.

To get the dosage down to something reasonable, it seemed that the ultimate compound, in the best tradition of the enzymologist, had to have an affinity for the transport system that secreted penicillin and yet be refractory to elimination by that system. This turned out not to be so, but it did give a conceptual direction that helped rationalize a solution to the problem. Probenecid, *p*-(dipropylsulfamyl)benzoic acid, had a renal clearance so low that within what was known about renal clearance in that day it did seem to fit the enzymologic thesis. What we did know about it was sufficient to permit us to anticipate the clinical dose in man precisely. Better known as Benemid, its place in management of gout was another story worth telling another time.

The purpose of this dissertation calls for "What Type of Structure-Activity Correlation Succeeds in Drug Design?" So let's get on to my favorite example.

My favorite example is the Diuril story, or the part that relates to your interests. A good orally active diuretic was needed, but it seemed like years went by before we were ready to tackle the problem. Getting ready was helped immensely by the advent of the practical flame photometer so that we could measure sodium and potassium easily. Getting ready required learning to control electrolyte excretion as well as urine volume. The key to success was the saluretic agent, a term we dreamed up to connote what we wanted.

I can hardly overstate the importance of methodology and a basic knowledge of the renal handling of salt and water. The prior literature was replete with diuretic assays in the rat and other simple screening methods, as in the mouse, based on the volume of urine excreted after drug administration compared with control values. Such methods at the time required little drug, were simple, and could be handled statistically. It turned out later that in such a rat assay, chlorothiazide is not impressive and might well have been missed. We definitely would have missed ethacrynic acid altogether. The dog protocol that we worked out required rather more drug, required a great deal more analytical effort and gave much more insight into what was going on in the animal. It was not deliberately complicated except in the sense of giving greater insight into the changes induced by the drug. Statistical control was impractical, and so we developed a simple EST assessment of the results of each clearance experiment. The individual letters, EST, meant, E, that the drug was or was not Effective in inducing a change, S, that the experiment was or was not Satisfactory technically and, T, that the agent was or was not Toxic. There were so many parameters to the test that had to interact appropriately, it was not too difficult to judge from a clearance experiment in one or two trained unanesthetized dogs whether a compound was worth following up. In structure/activity work, the assay has to give reliable negative results as well as positive.

The prior literature gave us our leads as to where to start. It also helped to keep competitors off our back. The paper by Schwartz in the *New England Journal of Medicine* (1949), which showed that sulfanilamide could be natriuretic in man, was a practical summarization of what had gone before with regard to the carbonic anhydrase work by Krebs, Keilin and Mann, Marshall, and others. The general feeling that organomercurials acted by inhibiting renal sulfhydryl catalyzed transport was a still more slender reed. Both leads were enough in either instance.

The fact that sulfamoyl-substituted aromatic compounds could inhibit carbonic anhydrase seems to have been used entirely differently in different labs in their search for diuretics. In our instance, we established to our satisfaction the earlier generalization that a sulfonamide had to inhibit carbonic anhydrase to be natriuretic. Thereafter, we disregarded the comparative in vitro carbonic anhydrase inhibitory activity. Emphasis was placed instead on the results in animals, for we really wanted to increase sodium and chloride excretion, not sodium and bicarbonate. Still others seem to have placed emphasis on building up carbonic anhydrase inhibitory activity in their compounds in vitro, conserving the animal experiments to check important leads. Fortunately for us, competitively speaking, those who put greatest credence in the literature could not see how a self-respecting carbonic anhydrase inhibitor could increase both sodium and chloride excretion. We thought it was possible. Very early in that work we found that *p*-carboxybenzenesulfonamide increased sodium and chloride excretion, but weakly so. The fact that it was not very potent in dogs or man may have spared the observation, attention, and credibility it deserved when we published that finding in 1954.

Perhaps the next most significant observation in a long string of compounds was that, properly placed, two sulfamoyl groups on a benzene ring were better than one. As is frequently the case, the addition of a chlorine adjacent to one of the sulfamoyl groups increased activity, and, again, two were better. From this observation derived the compound, 1,3 disulfamoyl-4,5-dichlorobenzene, marketed later as dichlorphenamide, or Daranide. It retained a great deal of the conventional carbonic anhydrase features, but the anionic excretory effect was pretty well shared between bicarbonate and chloride excretion. The compound was potent, and it seemed a good place to stop, but it really wasn't quite what we wanted. What we thought were its renal and corporal distribution characteristics were not quite what we were looking for.

It was known that acetylating the aromatic amino group, as in acetazolamide, enhanced activity. This was tried for chlorodisulfamoylaniline. Under the conditions being used, it cyclized to give the corresponding benzothiadiazine. This bit of good fortune on Fred Novello's part yielded just what we wanted by way of saluretic and distribution characteristics. The very first renal clearance experiment in dogs conducted by my associate, John Baer, characterized the electrolyte and water excretory pattern induced by the compound beautifully. The compound, 6-chloro-7-sulfamoyl-1,2,4-benzothiadiazine 1,1-dioxide, was named chlorothiazide. You probably know it better as Diuril. There is a lot more to both the chemistry and the biology that went into this work than I have time to tell, but these were salient points.

There is a great deal to be learned from these three examples about the interaction between biology and chemistry or, more importantly, between chemists and biologists who undertake such a joint effort. However, I should like to recite a few more case histories briefly that are a little different but which still illustrate some salient points.

Getting a saluretic agent out of a carbonic anhydrase in-

hibitor didn't seem that far fetched to us. On the other hand, I was taught and accepted, uncritically, the thesis that histamine did not play an important role in allergy. Hence, I discounted the early French work on antihistaminic agents. Earl Loew, then at Park Davis & Co., "knew" that histamine was important in allergy. He was prepared to recognize the significance of the compounds leading to diphenhydramine (Benadryl) that George Revieshel sent him. They led, and we never caught up with the early flood of antihistaminics. Our contribution to this field came much later and was oriented differently.

We would never have set out to discover an aromatic secondary amino ganglionic blocking agent. Everybody knew that a ganglionic blocking agent had to have at least one and better two quaternary heads. But when Clement Stone put the first aminoisocamphane into his cardiovascular experiment, he didn't need any help to appreciate the significance of what he had found. The second or third compound in the series that Karl Pfister and his associates sent to Stone ,(3-methylaminoisocamphane) was marketed as mecamylamine or Inversine. It was our entree to the field of antihypertensive therapy.

The beginnings of the story of my associates' involvement with coccidiostats, drugs to treat coccidiosis, goes back to the conviction of a veterinarian, David Green, that a sulfonamide active in malaria should be tried in chickens experimentally infected with coccidia. The chemist who believed him and who supplied the compounds was Max Tishler. Starting with sulfaquinoxaline, a whole series of interesting compounds useful for coccidiosis were developed under the supervision of a fine pair of biologists, Ashton Cuckler and Walther Ott. This work was done on a scale that ultimately only a computer could handle. The amounts of material employed in the biological assays was to the chemists' liking and the end point, life or death of infected birds, assured a better than usual initial transposition from laboratory to clinical conditions.

I suppose that if our chairman had wanted a chemist's idea of "What Type of Structure-Activity Correlation Succeeds in Drug Design," he would have asked a chemist to give the talk. All through this dissertation I have been more inclined to the biologist's view. But where the views of the chemist and the biologist can be identified with success, there is a common denominator. Tishler used to say that to be a success a project needs to have a champion. I imagine he still feels that way. Where structure/activity correlation is the key to success in drug design, I rather think there ought to be two champions for the project, one chemist and one biologist.

Each champion needs to bring a lot of special know-how to the project. It is a rare situation where the chemist knows enough biology or a biologist knows enough chemistry to do the whole job where structure/activity decisions need to be made. The chemist will disagree with this implied limitation to his virtuosity, but the biologist is usually not inclined to dominate the interaction at a technical level. Where structure/activity correlations are most apt to succeed is where a high order of respect and capability exists between the two champions of the project. Capability won't do it. Certainly just a pleasant ability to work together isn't enough. The likelihood of success increases with the expertise and conviction each can bring to the project. Expertise, discernment, and conviction are more to be sought than long training and a comprehensive knowledge of the literature. Without discernment, knowledge of the literature can be a hazard instead of a help. Without conviction, direction is easily lost.

Chemists and biologists are not natural bed fellows, they have to learn to live together. By heritage they see and do things differently. The chemist must define his compound precisely to be believed. The biologist needs to generalize the conditions under which an aberration or modulation of function can be induced reliably. Who ever heard of a statistical treatment of the melting point of a compound? Who would have suggested such a thing? What biologist would accept data on an experiment conducted in a single mouse or one elephant, for that matter. What chemist starts out to make more of a compound than he thinks he needs? What biologist ever asks for so little? What chemist can resist sending as many compounds per week as there is biological capacity to study them? What biologist wouldn't rather have time to think about what he is doing?

Usually the biologist would prefer to develop a background of information making use of established reference compounds. Unless the chemist can throw in a few other compounds from the literature, he may become part of the problem. The biologist, but not the biochemist, usually distrusts in vitro biological data as being too remote from end application to be reliably transposed to the clinical situation. The chemist loves such assays for they are direct. They frequently can be done in greater number and they usually require little compound. The biologist is frequently more inclined toward experiments in large animals, like dogs, that use up lots of the chemist's hard earned compound. And so, the daily adjustments of each to the other need to be made.

The way to success in structure/activity work is less sure than the way to failure. The way to failure is for either party, chemist or biologist, to abrogate responsibility for or fall short of the part he should bring to the joint effort.

For structure/activity work to be successful, there should be a clear definition of the biological objective in physiological terms rather than diagnostically. Where this can best be done, the advancement of therapy has been most satisfactory. Where we know least about the physiological basis for the signs and symptoms that characterize a disease entity, progress has been mostly slow or by happenstance. Hence, my reiteration of the need for the very best expertise and insight or discernment by the biologist as to what is going on. He does not necessarily need to know how or why at a molecular or subcellular level. However, such hypotheses evolved at that level certainly can help to give direction and motivation and even success when the basic design of the biological system is sufficiently forgiving.

I have spent a lot of time on especially the biological aspects of success, but the likelihood of success in structure/activity studies is only as good as the chemistry once a lead is at hand or an approach to synthesis is conceived. At this point, I should be listening instead of talking for seldom have I been able to interject into their thoughts anything useful that the chemists have not anticipated. What I envy is the feel that the really good medicinal chemist develops for how to introduce alteration in a molecule that will give it the subtle change in distribution or effect that the biologist wants. There are many ways of conceiving and estimating the interaction of physical attributes that lend a greater precision to the chemists' efforts, as I alluded to half facetiously but wholly enviously in my opening remarks. You know these better than I.

In a dinner speech before the Western Pharmacology Society a few years ago, I boldly said that we know how to discover new drugs. I think that is true even though we are not successful in doing so, much of the time. We are successful where the biologist can define precisely at a laboratory level and in physiological terms exactly what needs to be accomplished at a therapeutic level, be it for animal or man. If he can do that part, then the capable chemist who shares that confidence of success will very likely make the end objective, a useful new drug, come true.