University of Strathclyde Glasgow

Blue Sky Research:
Is it worth it?

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A cynic, perhaps a misguided politician, might ask whether we would be any worse off if certain academic research had not been carried out. This is a perverse question and deliberately misrepresents the issues. The thing about research, as Albert Einstein remarked, is that if we knew what the outcomes were going to be it wouldn't be research. Still less can we be sure in advance what research might turn out to be translatable into commercially or publically useful outcomes. In Victorian times, Michael Faraday was challenged that his research on electrical phenomena would not lead to anything important. So it's not a new question but one that periodically recurs. Perhaps these days it's the inherent risk that some people don't like but you don't solve societal problems simply by moving money around banks and funds. Buying a future in terms of research spending is a better bet than spending a fortune on risky futures!

But that's only my opinion. Is there any evidence that the cumulative output of research over the last 50 years has been valuable? Some sort of economic and social science research could be proposed to attempt to answer this question, the answer to which of course depends upon what you mean by valuable, opening a further possible dispute. With this in mind and the contents of my previous e-books in mind, I thought that I would attempt some sort of assessment myself by looking at things that have contributed to my research at the University of Strathclyde in previous e-books looking for new medicines (see 'New and effective drugs? Yes please, but where from?; It's a question of balance). The scientific fields that our work engages include organic chemistry, medicinal chemistry, chemical biology, pharmaceutical sciences, and many biological sciences leading into medicine. It's a sort of international continuum to which different labels have been attached, rather like the colours of the rainbow; continuing that analogy, I like to think of chemistry as the green bit moving through to medicine at the red end of the spectrum.

Very well, so what measures can we use to identify significant research that has influenced our work? There have been thousands of papers that we consulted in greater or lesser detail over the years. Some have been pointers to what we should do, some have helped explain our results, and still others have given us specific protocols to follow. These papers, like ours, have been set in the international continuum of sciences that I identified. They would not be recognized as significant except by specialists in the fields concerned. A more publicly recognizable identification of important scientific research comes from accolades to scientists of which the Nobel Prizes are probably the best known as recognizing the peak of scientific achievement. This is a good group to look at for several other reasons. Firstly, Nobel Prizes have a substantial history going back to the beginning of the 20th century. Secondly, the scientific fields covered include chemistry, physiology, and medicine, all of which overlap with my international rainbow continuum of research. Thirdly, their primary purpose as expressed in Albert Nobel's will is to recognize the scientific achievements of fundamental discoveries that have made an impact over a period of time. This is an ideal definition for my purpose because it directly takes on the challenge of the value of basic scientific research introduced in my first paragraph. Moreover I'm writing this as a scientist who has been arguably more concerned with translational studies than basic science.

I therefore went through the list of Nobel Prizewinners and picked out those whose research has contributed to the basis of what we have done. This means identifying anyone in the international continuum whose work has conceptually or practically been significant in our efforts to obtain new drugs for infectious disease and diseases of imbalance, including cancer, inflammatory disease, and central nervous system disorders. At the risk of repetition of the previous e-books, specifically we are

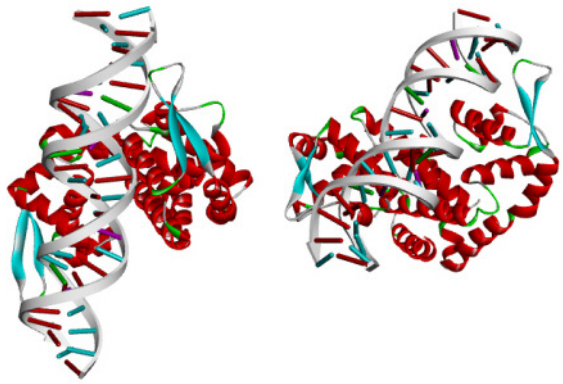

Figure 1. An illustration derived from X-ray crystallography of DNA from *Staphylococcus aureus*, one of our targeted organisms for antibacterial activity, (grey ribbons and attached sticks) with an associated protein fragment (red and magenta ribbons) prepared from 4lll.pdb (PDB Protein Structure Bank)

currently investigating anti-infective compounds to treat bacterial and parasitic infections in humans and animals; our most advanced compound has successfully completed a phase 1 clinical trial for treating *Clostridium difficile* infections and several others have achieved proof of concept in particular for treating African Animal trypanosomiasis. Compounds to treat inflammatory disease are similarly effective in models of arthritis, asthma, lupus, and lung fibrosis, for example. When cancer is added to this list you have a good idea of the potential impact of what we are doing.

The definition of what has been significant for us is a little fuzzy but I counted over 50 Nobel Prize winners whose work arguably matters at first sight. Let's not be fuzzy and look at some specific examples of Nobel Prizes and connect the work for which the prizes were awarded to what we have done. A good way to do this is to go step by step through the stages of discovering a new drug. I'll make one initial assumption, namely that a disease has been characterized by a clinician sufficiently well that it will be possible to recognize whether a new drug is doing any good or not. In what we've achieved so far, we are only just into human testing (clinical trials) but every example has reached a positive conclusion in animal models of the human (or other animal) disease. In reviewing the relevant Nobel Prizes, it is important to realize that the date of the prize is almost always many years following the discovery for which it is awarded. This is a necessary feature of the Nobel scheme; time has to elapse so that the impact of the key research can be clearly understood.

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Choosing the approach

With a disease identified, based upon its characteristics it is necessary to have a way to attack it, a biology-based hypothesis that connects a specific intervention by a drug to a potentially beneficial outcome for a patient. With respect to infectious disease, perhaps the person who made the most important written contribution to drug discovery was Adrien Albert, who in his remarkable book 'Selective Toxicity' (1951, and many later editions up to the 1980s) set out the principles connecting chemical structure and anti-infective drug activity. The heart of the matter is summarized in the title, selective toxicity, which emphasizes the obvious but critical point that an anti-infective drug should be toxic to the infective agent (bacterium, fungus, parasite, virus etc.) but not to the patient. We need a difference between the biology of the infectious agent and the host; this is exactly what the earliest antibiotics had, although with different origins. Prontosil (Domagk, 1939) was the prototype sulfonamide antibiotic and it acts upon a biological pathway that humans (and other animals) do not have, the biosynthesis of the folic acid vitamins. Penicillins (Fleming, Chain, Florey, 1945) inhibit the formation of the bacterial cell wall, a structure not found in higher organisms, so that it becomes fatally weak. Streptomycin (Waksman, 1952) inhibits bacterial protein synthesis by binding to the bacterial ribosome, the biological apparatus

Nobel Prize Winners whose work is significant to drug discovery at Strathclyde

- Gerhard Domagk (1939) for the discovery of the antibacterial effects of prontosil.
- Alexander Fleming, Ernst Chain, Howard Florey (1945) for the discovery of penicillin and its curative effect in various infectious diseases.
- Selman Waksman (1952) for his discovery of streptomycin, the first antibiotic effective against tuberculosis.
- James Black, Gertrude Elion, George Hitchings (1988) for their discoveries of important principles for drug treatment.
- William Campbell, Satoshi Omura (2015) for their discoveries concerning a novel therapy against infections caused by roundworm parasites.
- Tu Youyou (2015) for her discoveries concerning a novel therapy against Malaria.
- Alfred Gilman, Martin Rodbell (1994) for their discovery of G-proteins and the role of these proteins in signal transduction in cells.

responsible for protein synthesis. Humans also synthesize proteins using ribosomes but the ribosomes have different structures in detail from those of bacteria, hence selective toxicity arises.

Of these, the streptomycin case is the closest parallel for our antibacterial drugs. The Strathclyde compounds are minor groove binders for DNA (MGBs) and, of course, all forms of life that we know have DNA. We therefore rely upon differences in detail in the handling of DNA between bacteria and humans to provide the necessary selectivity. It's not known *a priori* that selectivity can be adequately achieved, but experiment has shown that it is the case. Accordingly our leading compound is now awaiting its Phase 2 clinical trial having successfully completed Phase 1 in a study sponsored by our partner company,

Figure 2. A branded box containing tablets of one of Black's drugs that contributed to his Nobel Prize, Tagamet (cimetidine)

MGB Biopharma. A more direct connection with streptomycin, however, is that we are working with partners in South Africa to develop an MGB active against streptomycin resistant strains of TB. The significance of infectious disease today has been emphasized by the award of a further Nobel Prize in this field (Campbell and Omura, and Youyou, 2015) for the discovery of the antiparasitic compound, avermectin (Campbell and Omura) and of a new antimalarial compound, qinghaozu (Youyou).

Turning to diseases of imbalance such as schizophrenia, which was mentioned in my last e-book, 'A Question of Balance', the story depended upon an understanding of the molecular target and the function of nerve cells. The basis for this was established by two Nobel Prizes, firstly in strategic terms by the demonstration that natural product analogues, including analogues of hormones, could produce useful, selective drugs (Black, 1988). Many naturally occurring hormones have multiple functions and in consequence a selective drug must act on preferably most strongly on only one function, in our case specific actions in the brain of schizophrenia patients. Black was the first person to demonstrate that this is really possible in his discovery of β-blockers and later of H2-antagonists. The second relevant Nobel Prize concerned the discovery of the molecular target for many drugs that treat imbalance, namely G-protein coupled receptors (Gliman, Rodbell, 1994). This discovery also related to the science underpinning Black's discovery of β-blockers to treat

angina and H2-antagonists to treat peptic ulcers. The citation 'for their discoveries of important principles for drug treatment' summarises that Black had demonstrated a new concept in drug discovery, a success shared by his co-laureates, Elion and Hitchings, although in a different way. Their contribution largely concerned relatives of cofactors, compounds that assist enzymes in catalyzing biological reactions, and was important mostly in antibacterial compounds. In our anti-schizophrenia project, we added a further dimension, namely deliberately targeting more than one G-protein coupled receptor at once. At the time this was a novel design feature and we did succeed in proving that the concept worked in an animal model of schizophrenia. Unfortunately, however, the compounds discovered were too toxic for development.

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Choosing the molecular starting points

There are several dimensions to many of the Nobel Prize discoveries identified (left). On the one hand there is the characterisation of disease to be treated and the specific biological strategy to be followed, in other words the mechanism by which the drug will successfully treat the patient. To obtain a drug, however, there has to be a starting point for the chemistry. In the long tradition of medicines and drugs, the first source of treatments was compounds made by plants and later compounds made by micro-organisms took the lead. The discoveries of Campbell and Omura belong to the first class and that of YouYou to the second.

In general, such natural products require some chemical modification in order to make them sufficiently stable, sufficiently water soluble, and sufficiently safe to make them into usable drugs. These modifications are the daily work of the medicinal chemist, to design and synthesize compounds that improve upon a template from nature. What Black, Elion, and Hitchings did was to show that the modification strategy could be applied to a much wider range of compounds than traditional natural products by operating on the very compounds that our bodies use to perform their complex, coordinated natural functions. Moreover, the nature of those modifications could be drawn from a much wider range of structural variations than had previously been considered possible. Factors such as physicochemical properties and the exact shape of the candidate drug compounds were brought into consideration for the first time.

These concepts, thoroughly developed subsequently by others, have been very important in medicinal chemistry at Strathclyde. Our anti-infectives projects have largely been based upon the structure of a natural product, distamycin, from a *Streptomyces* species but with an enormous range of structural modifications, some of which mirror the work of Black in his β-blockers discoveries. On the other hand, our immunomodulatory projects have been based upon a small component on the surface of an immunomodulatory protein synthesized by a parasitic worm to prevent its being eliminated by its animal host. This is a most unusual starting point for a drug discovery project but we've benefited also from applying imaginative structural design. The results form the basis of a new range of treatments for diseases such as arthritis, asthma, and lupus, for example.

Choosing the synthetic route

For a medicinal chemist, the two indispensable core skills are designing compounds and synthesizing them. It is no surprise that a significant number of Nobel Prizes in Chemistry were awarded for synthetic chemistry of different types. On the one hand, there are those concerned with the art and strategy of

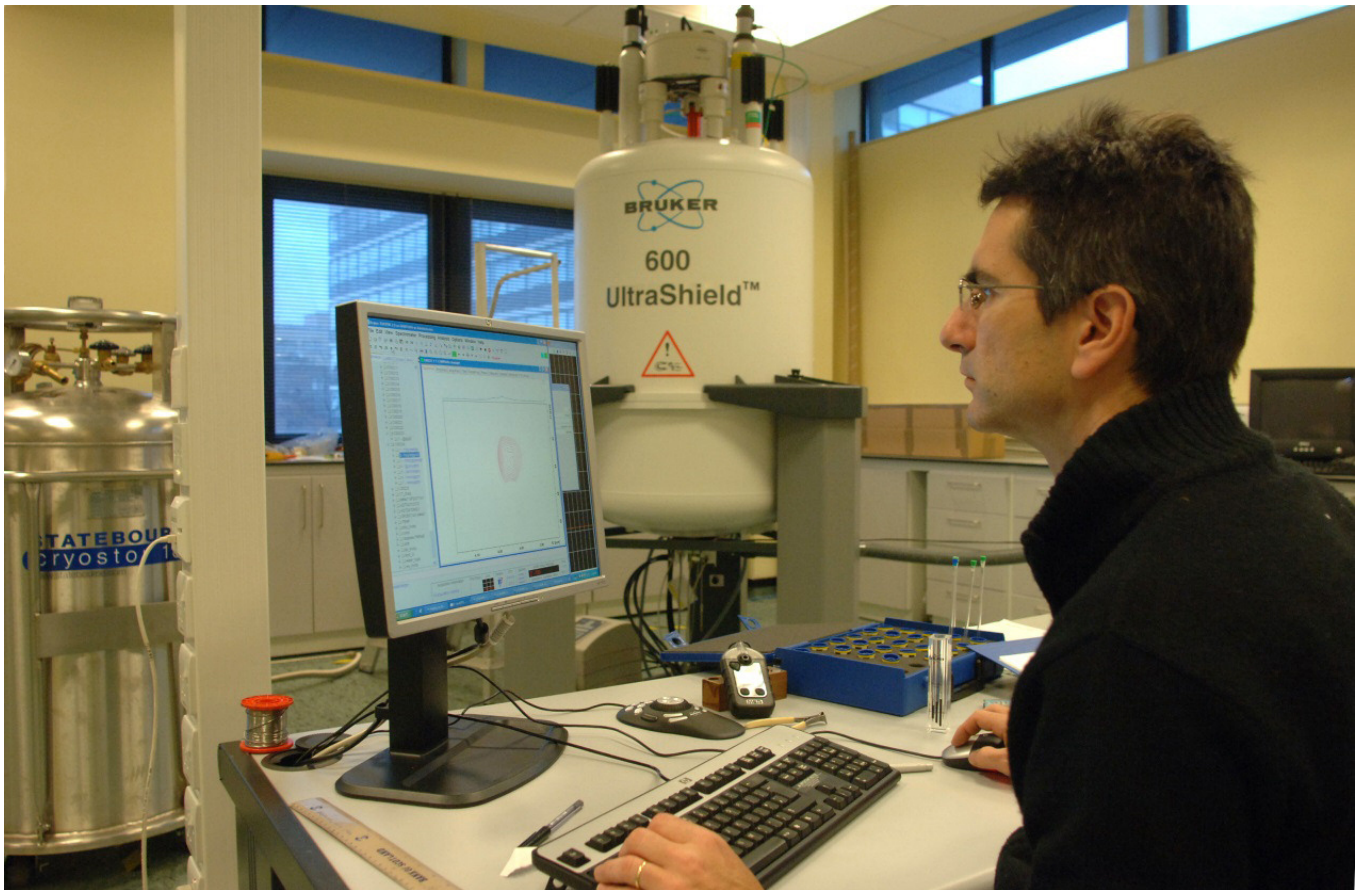

Figure 3. The high resolution NMR installation at the University of Strathclyde, Glasgow, at which the structures of antibacterial minor groove binders complexed with DNA were determined

synthesis, which affects every chemist involved in the preparation of sophisticated organic compounds, ourselves included. The prime mention for this contribution is Woodward (1965), who really showed how to think about things in a new way, and similarly Corey (1990).

The second aspect of synthetic chemistry for which Nobel Prizes have been awarded is the discovery of new reactions. New reactions quickly become routine but they have to be discovered in the first place. A large part of the work of synthetic organic chemistry is making new carbon to carbon bonds and several of the new reactions have used other elements to make this possible. In chronological order important examples in our work and that of many others is phosphorus (Wittig, 1979), boron (Brown, 1979), and palladium (Heck, Negishi, Suzuki, 2010). What characterizes all of these discoveries is that they have created families of reactions with wide scope and usefulness for many classes of compounds. In doing this the reactions make it possible to obtain compounds that otherwise would not have been readily available. Wittig-type chemistry has been used on a large scale to manufacture the material needed for the clinical trial of our antibacterial drug. Suzuki reactions have been extensively used in the discovery phase of an anticancer project in which we have to make many closely related compounds to establish a structure-activity relationship.

Others have introduced specialized methodologies that have created huge new opportunities. The technology of solid phase synthesis first introduced for peptides by Merrifield (1984) has proved to be the backbone of biopolymer synthesis including DNA and polysaccharides; without this much of modern molecular biology and chemical biology would simply not exist. In an earlier era, and feeding in to DNA synthesis, Todd (1957) showed how it was

possible to prepare the building blocks for DNA, nucleotides, thereby establishing the practical and conceptual basis for much heterocyclic synthesis. Moreover this work foreshadowed the contribution of Khorana (1968) who showed how the careful use of suitably designed protecting groups and chemical reactions made it possible to synthesize a gene for the first time; this work was a substantial part of the elucidation of the genetic code upon which so much modern biological science depends.

Something else without which modern research in chemistry and biology would not be possible is high resolution nuclear magnetic resonance spectroscopy (NMR). The range of applications is so vast and the level of detailed information available so great that it is impossible to imagine molecular science functioning without this technique. The foundation of modern high resolution methods by Ernst were recognized by the Nobel Prize in 1991. Apart from routine but sensitive characterization, in our work NMR methods have allowed us to determine the details of the how our antibacterial compounds bind to DNA and to obtain quantitative information about the stability of the complexes, all of which contributes to understanding their mechanism of action.

Showing how it works

When a new drug is moving towards registration and marketing it is very desirable to know how it works. Our understanding of these things depends upon the ideas we have about how molecules behave, our knowledge of how complex assemblies of molecules such as are found in nature behave, and the methods we have available to study these things.

In terms of molecular properties, some of the most important principles of reactivity and structure were described by Pauling (1954) whose ideas are firmly built into every organic chemist's conceptual model of the science. Ideas of shape and flexibility and rotation within molecules, that chemists collectively call conformation, are equally fully assimilated into the medicinal chemist's active mind (Barton, 1969). The concepts introduced by Pauling and Barton were very much part of the intuitive process that led to the design of our immunomodulatory compounds. The detailed working out of their ideas in general and their quantification have been developed by theoretical chemists, the impact of which has become huge as computing power has increased (Mulliken, 1966; Pople, 1998). Few medicinal chemical projects would proceed without some thought of theoretical chemistry or molecular modelling these days.

Like nuclear magnetic resonance, modern molecular science would be nowhere in its engagement with biology without X-ray crystallography. Some of the first work of medicinal chemical significance was recognized by the Nobel Prize awarded to Dorothy Hodgkin for her work on the structure of vitamin B12

Nobel Prize Winners whose work is significant in the synthesis of biologically active compounds

- Lord Todd (1957) for his work on nucleotides and nucleotide co-enzymes.
- Robert Woodward (1965) for his outstanding achievements in the art of organic synthesis.
- Gobind Khorana (1968) for ... interpretation of the genetic code and its function in protein synthesis.
- Herbert Brown, Georg Wittig (1979) for their development of the use of boron- and phosphorus-containing compounds, respectively, into important reagents in organic synthesis.
- Robert Merrifield (1984) for his development of methodology for chemical synthesis on a solid matrix.
- Elias Corey (1990) for his development of the theory and methodology of organic synthesis.
- Richard Ernst (1991) for his contributions to the development of the methodology of high resolution nuclear magnetic resonance (NMR) spectroscopy.
- Richard Heck, Ei-ichi Negishi, Akira Suzuki (2010) for palladium-catalyzed cross couplings in organic synthesis.

Nobel Prize Winners whose work is significant in understanding mechanism of action

- Linus Pauling (1954) for his research into the nature of the chemical bond and its application to the elucidation of the structure of complex substances.
- Max Perutz and John Kendrew (1962) for their studies of the structures of globular proteins.
- Francis Crick, Kames Watson, Maurice Wilkins (1962) "for their discoveries concerning the molecular structure of nucleic acids and its significance for information transfer in living material".
- Dorothy Hodgkin (1964) for her determinations by X-ray techniques of the structures of important biochemical substances.
- Robert Mulliken (1966) for his fundamental work concerning chemical bonds and the electronic structure of molecules by the molecular orbital method.
- Derek Barton and Odd Hassel (1969) for their contributions to the development of the concept of conformation and its application in chemistry.
- Walter Gilbert and Frederick Sanger (1980) for their contributions concerning the determination of base sequences in nucleic acids.
- Herbert Hauptman and Jerome Karle (1985) for their outstanding achievements in developing direct methods for the determination of crystal structures.
- Kary Mullis (1993) for contributions to the developments of methods within DNA-based chemistry [...] for his invention of the polymerase chain reaction (PCR) method.
- John Pople (1962) for his development of computational methods in quantum chemistry.

(1964). On a larger molecular scale we have the structures of the biological molecules with which our compounds engage, the prime example being the DNA double helix (Crick, Watson, Wilkins, 1962). The structure of proteins and protein complexes has had an equally large impact, the first of which, myoglobin, gave us the first detailed insight into the internal workings of a protein and started the journey towards an extensive understanding of protein structure and function that is behind almost every discussion of the mechanism of action of a drug that acts at a protein target (Perutz and Kendrew, 1962). Again like nuclear magnetic resonance, the development of methods and techniques have been important to make the speed of acquisition of data and its processing such that, given a good crystal, a protein structure can now be solved overnight, something that was hard to imagine even as little as 20 years ago (Hauptman and Karle, 1985).

In the field of nucleic acids, knowing the sequence of a nucleotide of interest is essential and today's studies were made possible by Gilbert and Sanger (1980) although further, more rapid methods capable of greater depth are routinely used now. These depend like much molecular biology and genetics and even forensics on the ability to prepare sufficient quantities of DNA to permit structure determination from traces of purified DNA by what is known as the polymerase chain reaction (PCR) developed by Mullis (1993). PCR and its variants have been used extensively in our studies of the mechanism of action of our antibacterial drugs in terms of their effects on the DNA of the bacteria that we seek to kill.

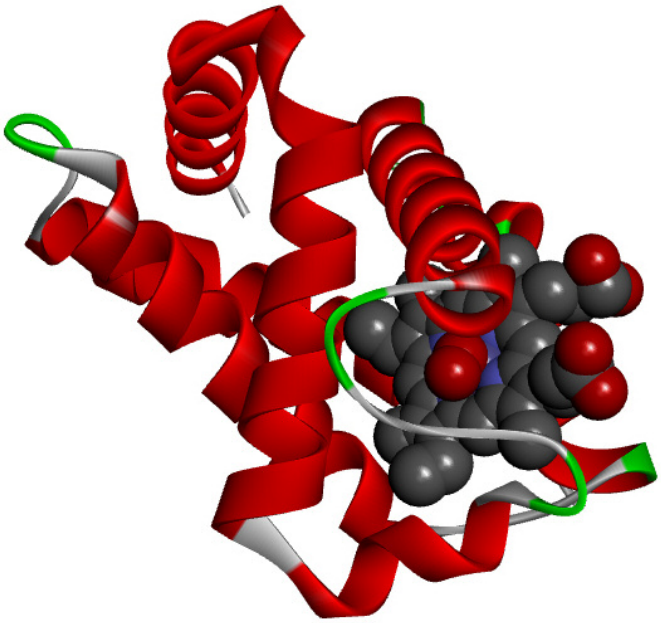

Figure 4. A section of a modern X-ray crystallographic structure of haemoglobin showing the site where oxygen binds to iron. Derived from pdb structure file 2DN1

Have I made my case?

If these discoveries have been important for us then they have been important for many other scientists too. I've cited 25 Nobel Prizes relevant to work that I've mentioned in previous e-books for Adjacent Government. If I had expanded the range to include other substantial projects that I have worked on there could easily have been as many more. There are as many for physiology or medicine as there are for chemistry. Modern medicinal chemistry absorbs all of these sciences as is needed to tackle the challenge of treating human and animal health better. There's not a new medicine that reaches the market that will not have been discovered with the benefit of discoveries recognized by Nobel Prizes, and indeed the many other awards that are made to distinguished scientists. We could not do the applied and translational work in medicinal chemistry without the prior basic science. Indeed in our academic environment we try to contribute to basic science in parallel with our drug discovery. That's one of the things that makes it so fascinating and so much fun. With a little luck and more than a little money, perhaps one day one of our discoveries will successfully treat a patient.

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