2008 Alfred Burger Award Address in Medicinal Chemistry

Discovery of Innovative Small Molecule Therapeutics

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Opening Remarks

I'm honored and privileged to receive the prestigious Alfred Burger Award in Medicinal Chemistry and to join the list of distinguished individuals who received this honor before me. I regard this award as a reflection and recognition for the efforts of my research teams at Wyeth Research, for their creativity and dedication over the years, and for the contributions of many talented partners and collaborators who have made it possible for me to receive this important award. Over the years I have benefited from the advice and wisdom of many inspiring mentors and role models and am deeply indebted to all of them.

I share with you my journey working in Drug Discovery at Wyeth at the four discovery research sites over the past 2 decades and reflect on the evolution in medicinal chemistry approaches that have been successfully utilized in discovering today's innovative drugs.

Introduction

Today's innovative drug discovery is costly and time-consuming, with very few novel therapeutics making it to the market place. Traditional medicinal chemistry approaches adopted during the 1970s and 1980s were focused primarily on analoguing of endogenous ligands and industry leads.[1] Chemistry was low throughput and done iteratively, driven primarily by biochemical observations derived from animal testing. In contrast, the past decade has witnessed an evolution in medicinal chemistry approaches wherein automation has been effectively utilized in the synthesis of large numbers of analogues (combinatorial chemistry) and in the rapid screening of large numbers of compounds (HTS[a]).[2,3]

At the turn of the century, subsequent to identification and characterization of large numbers of targets, the deciphering of the human genome led to an explosion in the "-omics" technologies. The assimilation of the resulting information and correlation of potential therapeutic targets with human diseases present tremendous challenges for drug research. Nonetheless, advances in technology have enabled the pharmaceutical industry to explore multiple medicinal chemistry approaches in support of chemical biology efforts and to identify leads and optimize drug candidates. These advances include improvements in structure-based design, integrating X-ray crystallography, computational chemistry, nuclear magnetic resonance spectroscopy, multivariate analysis, parallel synthesis, and early pharmaceutical profiling. Application of these techniques, coupled with the growing field of biosynthetic engineering, precise synthetic methods, and the use of high-resolution analytical tools, has also spurred renewed interest in natural-product-based drug research.

This lecture will give a brief overview of various medicinal chemistry approaches from the past to present that we utilized successfully to discover several marketed drugs and clinical candidates. These approaches include the following sections: (1) Ligand-Based Design, (2) Discovery of CNS Drug Candidates, (3) Multidimensional Lead Optimization, (4) Pharmaceutical Profiling, (5) Structure-Based Design, (6) Natural-Products-Based Drug Design, (7) Future Outlook.

Ligand-Based Design

Ligand-based design, also referred to as pharmacophore-based design, capitalizes on the presence of existing structural similarities between a set of compounds and active ligands. The sources of active ligands vary and include literature, patents, and in-house experimental data of earlier lead compounds. This approach usually provides a good starting point for the chemist where the selected set of compounds is often more active than randomly selected compounds. The power of the pharmacophore-based approach for lead generation lies in its ability to identify diverse sets of compounds potentially possessing a desired biological activity but having different chemical scaffolds. This approach has been used extensively in the discovery of CNS therapeutics.

Discovery of CNS Drug Candidates

Design of therapeutics for treatment of CNS disorders has evolved from the early serendipitous discovery of chlorpromazine and benzodiazepine to current approaches utilizing ligand-based design. Alterations in biogenic amine neurotransmitter levels in the brain have been implicated in the pathophysiology and pharmacotherapy of a number of neurological and psychiatric disorders.[4] Over the years, the biogenic amines have

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[a] Abbreviations: 5-HT1A, 5-hydroxytryptamine subtype 1A; 5-HT2, 5-hydroxytryptamine subtype 2; 8-OH-DPAT, 8-hydroxy-2-(di-*n*-propylamino)tetralin; AD, Alzheimer's disease; ADME, absorption, distribution, metabolism, and excretion; ADMET, absorption, distribution, excretion, and toxicity; AIDS, acquired immune difficiency syndrome; BACE, β amyloid converting enzyme; CNS, central nervous systerm; CoMFA, comparative molecular field analysis; CYP, cytochrome P450; D2, dopamine subtype 2; EPS, extrapyramidal side effects; HTS, high throughput screening; NCE, new chemical entity; NMR, nuclear magnetic resonance; SAR, structure−activity relationship; SPR, structure−property relationship.

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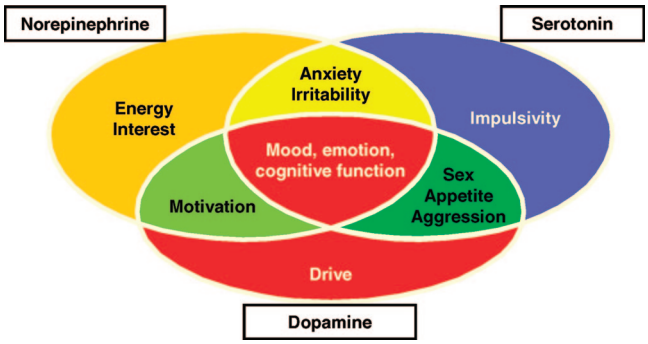


Figure 1. Biogenic amines as a starting point for drug design.

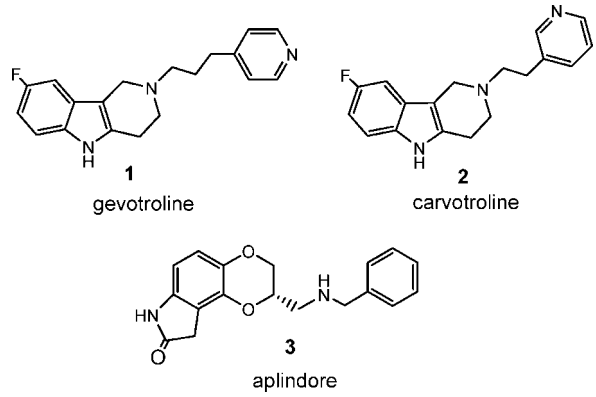


Figure 2. Structures of dopaminergic drug candidates.

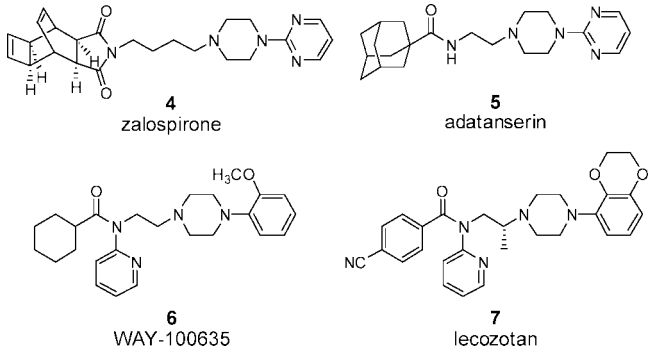


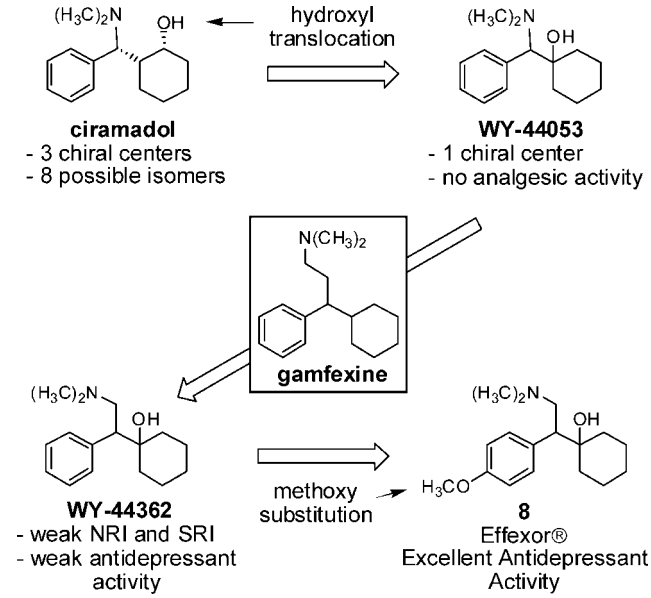
Figure 3. Structures of serotonergic drug candidates.

provided medicinal chemists with a solid starting point for their design of innovative therapeutics (Figure 1).

Dopaminergic Drug Candidates

The dopamine hypothesis of schizophrenia has prompted vast research in an attempt to achieve normalization of dopaminergic activity. Studies have shown that neuroleptic therapy with antipsychotic drugs with apparent specificity for the limbic as opposed to the striatal region of the brain may provide efficacy with a lower risk of extrapyramidal side effects (EPS).[5,6] Our early research efforts in this area focused on developing novel atypical antipsychotic drugs that exhibited weak or moderate antagonist activity at D2 receptors as well as 5-HT2 antagonist activity.[7] This work led to the design and discovery of two atypical antipsychotic agents: gevotroline **1** and carvotroline **2**. Both compounds demonstrated profiles in animal models of schizophrenia that suggested they might provide antipsychotic efficacy with reduced liabilities. For example, **1** and **2** blocked apomorphine-induced climbing in rats more potently than apomorphine-induced stereotypy and both compounds inhibited

Scheme 1. Structural Evolution of Effexor



conditioned avoidance responding in rats and monkeys without inducing catalepsy.[8,9] Compounds **1** and **2** demonstrated antipsychotic activity in schizophrenic patients in phase II clinical trials.[10,11] An alternative approach that we pursued for balancing the paradoxical dopaminergic activity in the cortical and limbic systems was to identify D2 partial agonists.[12] CoMFA-driven SAR studies directed toward discerning the D2 receptor agonist pharmacohore and topography identified two advanced series, the 7-hydroxyaminochromans (7-OH-AMC) and the 7-hydroxyaminobezodioxans (7-OH-AMB),[13] and ultimately led to the discovery of the D2 partial agonist Aplindore[14] (DAB-452, **3**). Compound **3** demonstrated potent dopamine D2 receptor affinity and intrinsic activity between those seen with full agonists and the antagonist aripiprazole in a variety of in vitro assays and in an in vivo model of dopamine agonism. We hypothesized that the level of intrinsic activity seen with **3** should prove useful in modulating dopamine activity in conditions such as schizophrenia and provide dopaminergic tone in

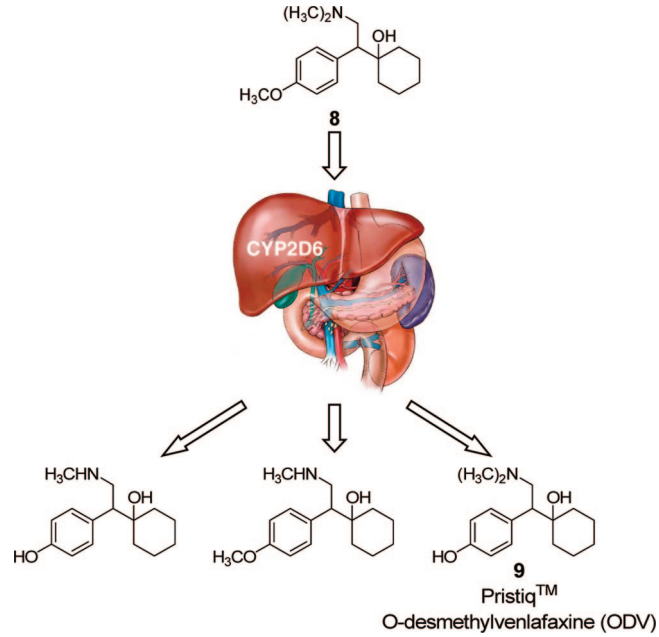


Figure 4. Major human metabolites of Effexor.

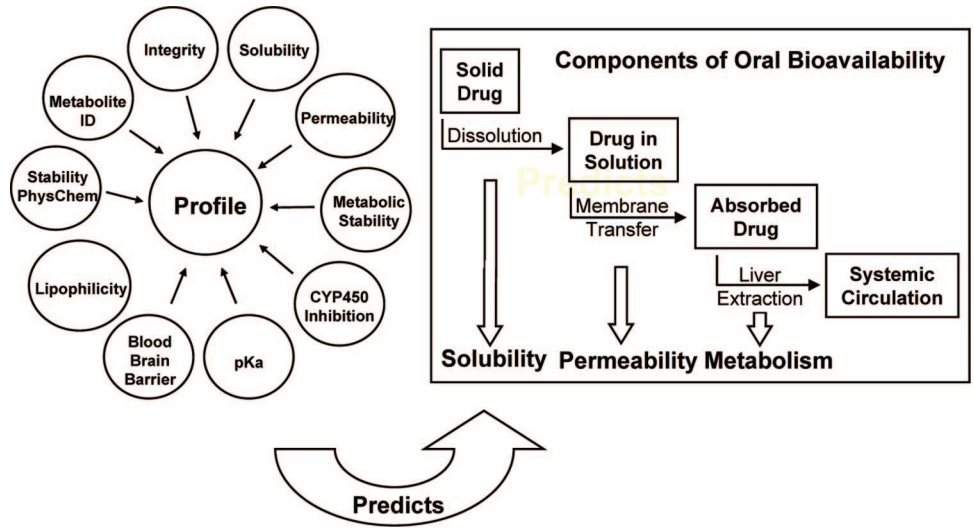


Figure 5. High throughput screening of druglike properties.

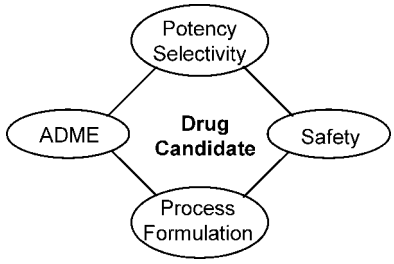


Figure 6. Multidimensional lead optimization.

conditions where dopamine levels are low, such as in Parkinson's disease. Compound **3** advanced to phase II (Figure 2).

Serotonergic Drug Candidates

The discovery of the aminotetralin 8-OH-DPAT, a potent selective 5-HT1A full agonist, facilitated the development of several 5-HT1A ligands.[15] Our efforts in this area led to the discovery of 5-HT1A partial agonist zalespirone **4** and mixed 5-HT1A/5-HT2 antagonist adatanserin **5**; both demonstrated anxiolytic activity in animal models and advanced to phase II for the treatment of anxiety disorders.[16−20]

Building upon structural features of **4** and **5** led to the discovery of a number of selective 5-HT1A antagonists, including WAY-100,635 (**6**) and lecozotan (**7**).[21] The lack of intrinsic activity of **6** in multiple assay systems has made it a widely used pharmacological tool, but its poor oral bioavailability limited its clinical potential. Compound **7** was efficacious orally in reversing cognitive deficits in monkeys and advanced to phase II for the treatment of Alzheimer's disease (Figure 3).[22,23]

Multitarget ligand-based designs have been successfully utilized in the search for effective antidepressant drug candidates. While the majority of antidepressant research in the 1980s focused on design of novel selective serotonin reuptake inhibitors (SSRIs), we focused our efforts on the design of multitarget serotonin/norepinephrine reuptake inhibitors (SNRIs).

We capitalized on the existing structural similarities between one of our earlier opiate analgesic leads, ciramidol, and antidepressants that were under clinical investigation.[24] Ciramidol was further optimized (Scheme 1) by reducing its chiral centers from three to one and incorporating an alkylamine pharmacophore in a three-step synthesis to afford Effexor (venlafaxine WY-45,030, **8**).

Compound **8** represented the first-in-class of SNRIs. It demonstrated efficacy in a broad range of patients including those that were difficult to treat. Since its launch in 1997 as Effexor XR, it has become the mainline therapy for major depressive disorders (MDD) and has been shown to have higher remission rates than SSRIs.[25]

The remarkable clinical efficacy of Effexor XR that has benefited over 15-million patients since its launch has prompted the search for a second generation SNRI. Consequently, Duloxetine was launched in 2004 and is reported to have more balanced affinities at the serotonin transporter (SERT) and norepinephrine transporter (NET). We observed that **8** is metabolized in the liver into three metabolites (Figure 4). One major metabolite, *O*-desmethyl venlafaxine (ODV) **9** is more bioavailable than Effexor and is also an inhibitor of both serotonin and norepinephrine reuptake.[26] Further investigation resulted in its FDA approval and launch in 2008 as Pristiq.

Multidimensional Lead Optimization

Drug discovery has undergone considerable changes during the past 2 decades as new enabling technologies have been introduced. These have provided us with new opportunities to increase drug discovery success and efficiencies on all levels. These new technologies not only assist in the optimization of biological properties of lead compounds but also allow the chemist to address pharmaceutical parameters and druglike physical properties earlier in the drug design process and on a much larger scale than was previously possible. In the past, selection and optimization of lead compounds and the selection of drug candidates were driven primarily by results from biological assays, but recently parallel druglike property optimization has added a new discovery dimension.[27−29]

Pharmaceutical Profiling. Structure−Properties Relationship (SPR)

Druglike compounds are defined as those that have sufficiently acceptable chemicophysical and ADME properties and sufficiently acceptable toxicity to survive through the completion of human phase I clinical trials. Drug properties have always been a prominent component of the development phase, where detailed studies are performed on formulation, stability, PK, metabolism, and toxicity. However, in recent years it is has become imperative to integrate drug property evaluation into the discovery process. This has been achieved by building

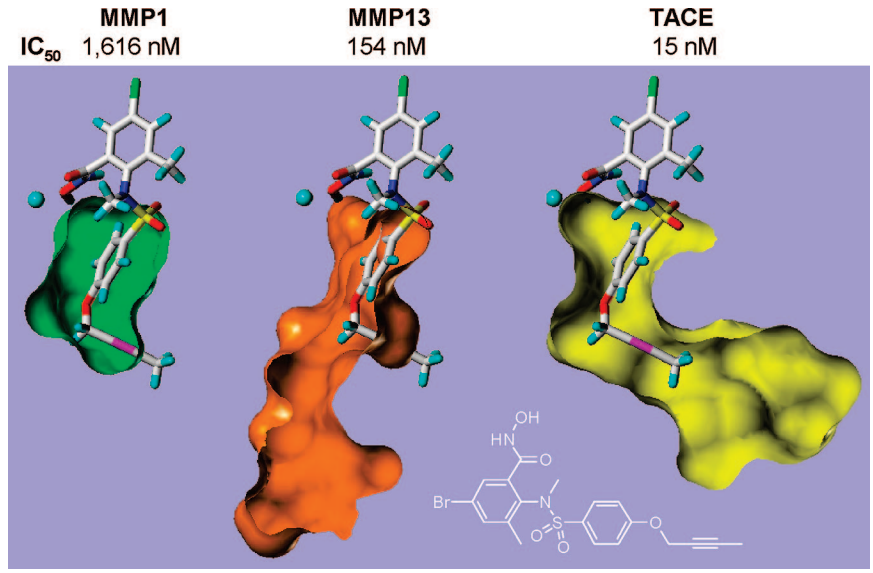
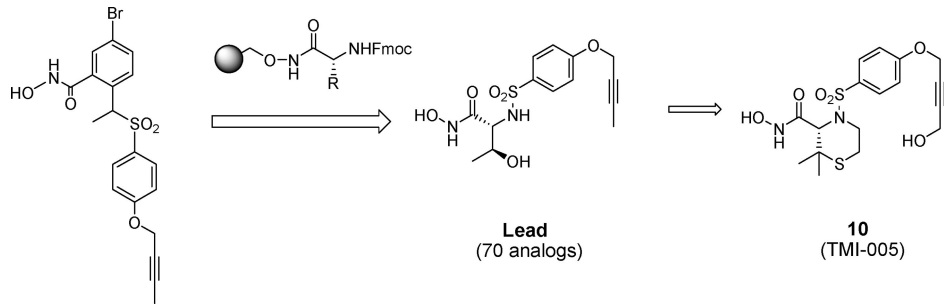


Figure 7. Comparing the active sites of MMPs and TACE.

Scheme 2. Evolution of TMI-005



capability for measuring ensembles of essential "druglike properties". This structure−property relationship (SPR) information complements traditional SAR information and is rapidly becoming an essential tool for medicinal chemists. It allows the chemist to address druglike properties earlier in the drug-design process as well as providing information that can help in the interpretation of results from in vitro and in vivo assays.

In many instances poor efficacy in in vivo models may be due to low concentrations in the plasma and target tissues. This low bioavailability can be caused by a number of physical properties, such as poor solubility, poor permeability and absorption, active transport, and low metabolic stability.[30,31] In particular, poor efficacy for CNS drug candidates in vivo may be caused by poor penetration of the blood−brain barrier (BBB) and/or low levels of free drug in the CNS. For CNS drug discovery, often the goal is to optimize unbound drug brain/plasma (B/P) ratios rather than total B/P ratios or total brain drug concentration.[32]

High throughput assays for physicochemical properties, solubility, permeability, lipophilicity, stability, and Pka-in vitro ADME (metabolism, transporters, protein binding, and CYP inhibition) and in vivo PK exposure, provided a wealth of data for the discovery scientists to make informed decisions (Figure 5). These assays complement the in silico approaches currently utilized to predict physicochemical parameters of molecules such as MetaSite.

Multidimensional optimization combining the physicochemical properties data with biological activity at the target site added tremendous value to the selection of development track candidates. The process is further guided by favorable PK and safety profiles and finding the suitable formulation for initiating first-in-human trials (Figure 6).

Structure-Based Design

Rational drug design involves the use of three-dimensional information resulting from interactions of small molecules with biomolecules utilizing techniques such as X-ray crystallography and NMR spectroscopy. This approach has been successfully utilized in the discovery of several drug candidates, particularly the design of enzyme inhibitors.[33] In an effort to find effective therapies for treatment of inflammatory diseases, inhibition of TNF-α converting enzyme (TACE) represented an attractive drug discovery target for reducing the circulating level of the proinflammatory soluble protein TNF-α. Sulfonamide hydroxamate TACE inhibitor leads were further optimized for potency and selectivity over matrix metalloproteases (MMPs) utilizing information derived from X-ray cocrystal structures. The S1′ pocket of TACE consists of an initial region resembling the shallow S1′ pocket of MMP-1 in size and depth. The rest of the TACE S1′ is no longer extended linearly as in MMP-9 and MMP-13; rather, the extended channel is almost perpendicular to the S1′ pocket, directed toward the S3′ surface (Figure 7).[34]

Given this information and by use of a solid phase combinatorial chemistry approach, a series of phenoxyacetylene hydroxamates was synthesized. Optimizing these leads for potency and selectivity helped define the SAR requirements for the TACE binding site.[35] Subsequent optimization for PK and in vivo efficacy through parallel synthesis led to the discovery of TMI-005 **10**, a propargylic hydroxylhydroxyamic acid that

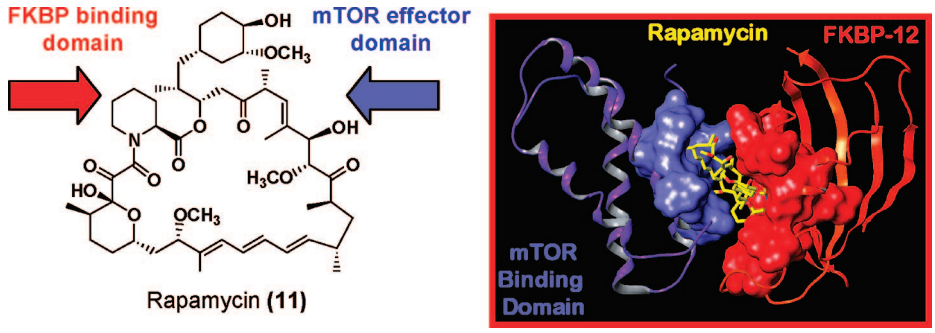
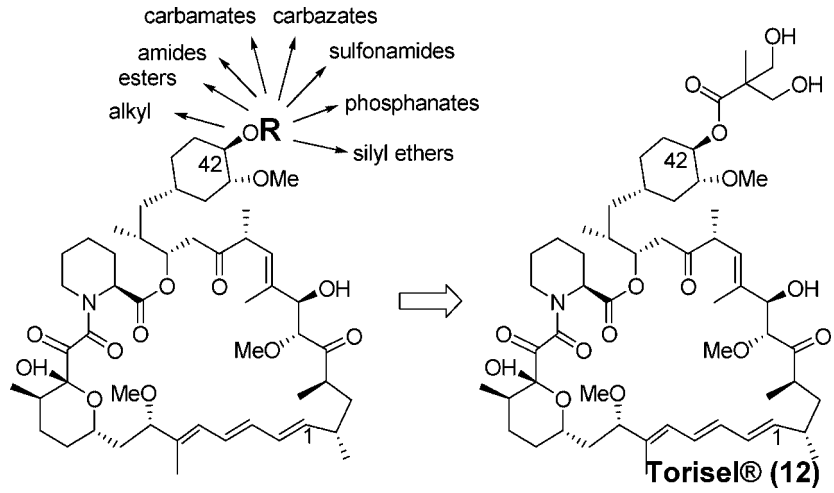


Figure 8. Binding of rapamycin to FKBP and mTOR42.

Scheme 3. Functionalization of C-42 Alcohol Led to the Discovery of Torisel



advanced to phase II for treatment of rheumatoid arthritis (Scheme 2).[36]

Natural-Product-Based Drug Design

Natural products and their derivatives have historically been invaluable as a source of innovative therapeutics. Natural-products-based drug discovery reached its peak during the 1970s and 1980s. Of the small molecule NCEs introduced to the market between 1981 and 2002, roughly half (49%) were natural products, semisynthetic natural product derivatives, or synthetic compounds based on natural product pharmacology.[37,38]

Despite this success, pharmaceutical research into natural products has encountered significant challenges during the past decade. In recent years, the pharmaceutical industry has placed low emphasis on natural-product-based drug discovery and several big pharma companies have exited natural product research. The decline in natural product research could be attributed to several factors such as lack of compatibility of traditional natural product extract libraries with HTS, demand on reducing hit-to-lead cycle time which favors screening of small molecule chemical libraries, waning interest in infectious disease research which is usually benefited from screening of natural products, development of combinatorial chemistry, and challenges in gaining access to biodiversity.

Advances in technology have enabled natural-product-based drug discovery to operate on a more rational basis, and this has spurred renewed interest in natural products research which is now feasible using precise synthetic methods and high-resolution analytical tools.[39] Genetic engineering of biosynthetic pathways of biologically active natural product scaffolds can provide new starting points for optimization of these privileged structures.

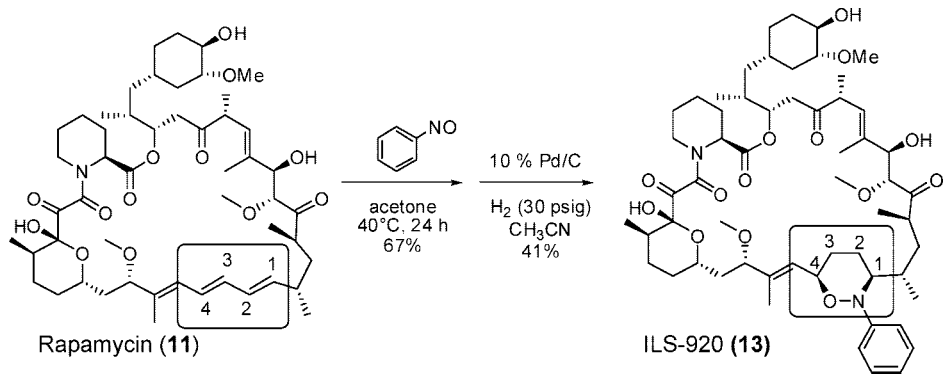
Natural-product-based drug discovery was successfully applied to rapamycin **11**, a novel immunosuppressant natural product with a unique mechanism.[40] It binds to the effector protein FKBP and forms a complex that binds to another effector protein, m-TOR[41] (mammalian target of rapamycin, Figure 8). Rapamycin was marketed in 1999 as Rapamune for treatment of transplantation rejection.

We embarked on a non-HTS approach looking at our rapamycin equity in order to expand its therapeutic utility. Applying a semisynthetic strategy to the C-42 secondary alcohol functionality led to the discovery of CCI-779 **12**, a cell cycle inhibitor with potent antitumor activity that was marketed in 2007 as Torisel for treatment of renal cell carcinoma[43] (Scheme 3).

The discovery of Torisel will be the subject of an upcoming case study perspective article.

It is well established that immunophilins ligands such as FK-506 demonstrate neuroprotective activity in animal ischemia models. A key event in this process was thought to be binding to FKBP-12, which can trigger a cascade of events, although the exact mechanism is not fully known.[44,45] One hindering factor to using these compounds as neuroprotectants has been their immunosuppressant activity. However, it is possible to achieve neuroprotection using immunophilins without immunosupression.[46−48] Efforts aimed at exploring the role of these nonimmunosuppresive immunophilin ligands in neuroprotection revealed that, while binding to FKBP-12 was still a key step, formation of a ternary complex with calcineurin may not be responsible for mediating neuroprotection and neuroregeneration.

Scheme 4. Synthesis of ILS-920 (**13**)



Our efforts in designing non-immunosupressant neuroprotectant immunophilins focused on the pharmacophore that binds to the m-TOR effector protein. Structural manipulation targeting the C1−C4 triene of rapamycin utilizing Diels−Alder chemistry with a variety of dienophiles led to the synthesis of novel nonimmunosuppressant neuroprotective rapamycin derivatives. One of these, the semisynthetic adduct ILS-920 **13**, lacked immunosuppressant properties and demonstrated good brain penetration. It was synthesized in a three-step synthesis by reacting rapamycin with nitrosobenzene followed by hydrogenation of the six-membered cyclic adduct (Scheme 4).

Compound **13** promoted survival of E16 primary rat cortical neurons in culture and stimulated neurite outgrowth in that system (Figure 9).[49] The compound demonstrated neuroprotection in a rat transient mCAO focal ischemia model in a dose-dependent manner (Figure 10).[49] In that model, animals treated with **13** showed reduced neurological deficits compared with placebo-treated animals, as measured by sensorimotor performance. In a permanent focal ischemia model, that improvement in sensorimotor performance compared to placebo lasted up to 3 months. Taken together, these data suggest that ILS-920 not only functions as a neuroprotectant but also promotes functional recovery. These exciting results will be featured in an upcoming manuscript.

Graziani et al. have provided evidence that that the neuroprotective and neuroregenerative activities of **13** may involve binding to the effector protein, FKBP-52. In addition, inhibition of L-type calcium channel currents may contribute to the compound's neuroprotective activity.[50]

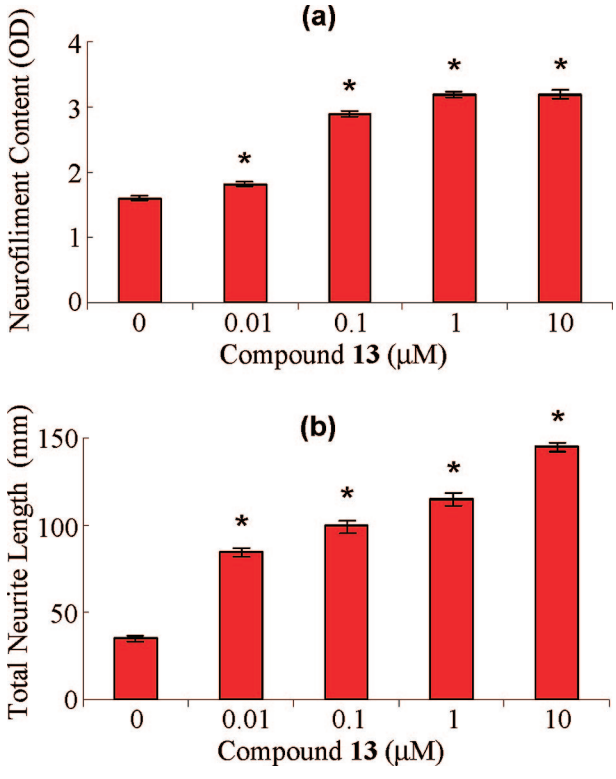


Figure 9. Neuroprotective/neuroregenerative activity of compound **13** on E16 primary rat neurons in culture: (a) effect of **13** on neuronal survival; (b) compound **13** promoting neurite outgrowth.

Closing Remarks and Future Outlook

During my 26-year journey working in the pharmaceutical industry, I have experienced vast changes in medicinal chemistry approaches where new NCE drug candidates were discovered. Shifting emphasis from optimizing biology of synthesized molecules into optimizing druglike properties and the ADMET profile led to reduction in attrition rate of compounds in development. Current and future approaches include nontraditional approaches to the discovery of drug candidates. This will include bridging the gap between small-molecule and protein drug candidates by applying multiplatforms (small molecules, vaccines, and proteins) in an integrated strategy to tackle a given disease target. We have adopted this approach in applying our research capabilities to improve upon current approaches to AD treatment by utilizing multiple research platforms.

In addition to developing drugs that more effectively control AD symptoms, we are expending significant resources on novel approaches to slow or halt the progression of the disease. We are using three technology platforms in our AD development efforts.

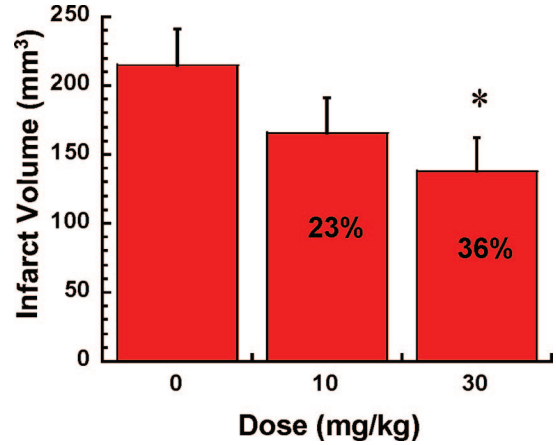


Figure 10. Compound **13** reduces infarct volume in a rat transient mCAO model.

Our small molecule approach involves designing γ-secretase and BACE inhibitors to inhibit formation of β-amyloid protein (Aβ). We have also taken a biopharmaceutical approach that involves designing recombinant proteins, vaccines, and human recombinant antibodies that stimulate the body to clear both soluble Aβ and deposited β-amyloid (plaque) from the brain.

Nontraditional approaches to natural-products-based drug discovery are now being adopted such as the use of fractionated libraries which result in broader assay compatibility and fewer interferences and lead to faster identification and isolation of active leads. Future natural products drug discovery leads not only will capitalize on semisynthetic approaches but will focus on a "customized natural products approach" wherein new lead molecules once thought to be inaccessible by semisynthesis are generated through biosynthesis and genetic engineering. We successfully adopted both strategies that resulted in the discovery of the anticancer drug Torisel, neuroprotectant drug candidate ILS-920, and several new natural products derivatives currently in development.

Fragment-based lead discovery is another emerging approach that is complementary to the traditional HTS strategy that is commonly used for hits (leads) identification. Fragment-based screening offers several advantages over HTS, such as screening smaller numbers of compounds (hundreds as compared to thousands in HTS). Compounds screened are usually of smaller molecular weights than compounds screened in HTS, and identified hits in fragment screening usually achieve higher binding efficiency but weaker potency than those identified via HTS. It is generally accepted that the fragment-based approach emphasizes efficiency and design while the HTS emphasizes affinity and number.[51] It is also accepted that the fragment approach is more appropriate at the early stages of the optimization process given that rigorously defined criteria are established for the selection of targets subjected to this approach due to the constraint on X-ray crystallography and NMR resources.

As more innovative new drugs acting at novel targets advance through development, it becomes increasingly important to establish a correlation between preclinical efficacy in animal models and efficacy in humans. Translational medicine is rapidly emerging in many biomedical research institutions as a tool to bridge this gap between preclinical and clinical studies, often referred to as "from bench to patient bedside". Medicinal chemists are playing a pivotal role in the discovery of biomarkers, from radiolabeled synthesis efforts in imaging studies to the development of sophisticated instrumental analysis methods and biochemical assays in metabolomic investigation that are used to measure biomarker levels in biological fluids.

Thus, while drug discovery hurdles remain many and high, the future holds a great promise for this industry. We will continue discovering major breakthrough therapies that will address many unmet medical needs, such as disease-halting drugs for the treatment of Alzheimer's disease, drugs to eradicate AIDS, and drugs to attack resistant cancers, treat strokes, MS, and other debilitating diseases.

Acknowledgment. The author is indebted to many Wyeth researchers who are responsible for the discovery of these many breakthroughs and first-in-class drug candidates. Sincere thanks go to my colleague Wayne Childers not only for providing constructive feedback and suggestions to the content of the presentation and this article but also for his dedicated tireless efforts in medicinal chemistry during the past 20 years. I also express my deep appreciation and thanks to Cynthia Tagliaferi for her efforts in preparing this manuscript.

Biography

Magid Abou-Gharbia received his B.Sc. (1971) and M.Sc (1974) degrees from Cairo University in Pharmacy and Pharmaceutical Sciences and his Ph.D. in Organic Chemistry from the University of Pennsylvania (1979) under the direction of Professor Madeleine Joullie. After an NIH postdoctoral fellowship at the Medical School and Chemistry Department at Temple University he joined Wyeth in 1982. He spent 26 years working in many therapeutic areas where he and his research team have made numerous contributions in medicinal chemistry that resulted in the discovery of several innovative marketed drugs, few of which are described in this article. In September 2008 he joined the School of Pharmacy at Temple University as Professor of Medicinal Chemistry and director of the Center for Drug Discovery Research (CDDR).

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