



**Trip Report:
12th International Annual Molecular
Medicine Tri-Conference
San Francisco, California
April 20– 22, 2005**

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Aruna Sambandam, Ph.D. and Larry Yet, Ph.D.**

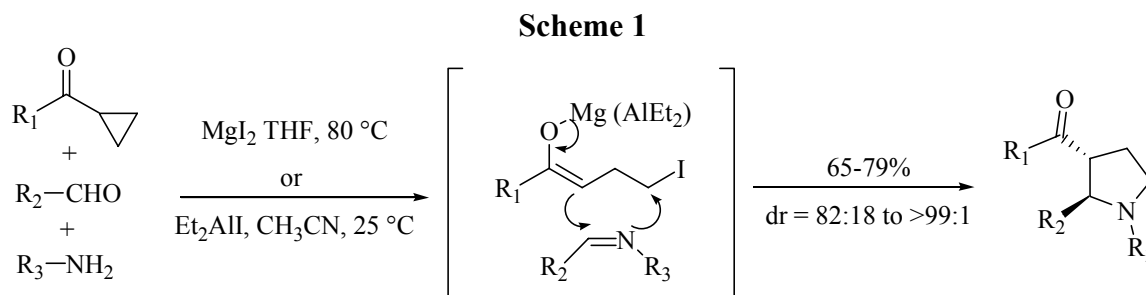
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***Abstract.** Cambridge Healthtech Institute's "Twelfth International Annual Molecular Medicine Tri-Conference" was held in San Francisco, CA, on April 20-22, 2005. About 2200 attendees from both biotech and pharmaceutical companies attended this conference. This symposium contained featured five concurrent tracks: Track 1: Pathway Analysis; Track 2: R & D Strategies; Track 3: Mastering Medicinal Chemistry; Track 4 Integrative Preclinical Development; Track 5: Molecular Diagnostics. This report highlights select material from information presented in seminars from Track 3: Mastering Medicinal Chemistry.*

"A Combinatorial Scaffold Approach Toward Pharmacophore Mapping,"

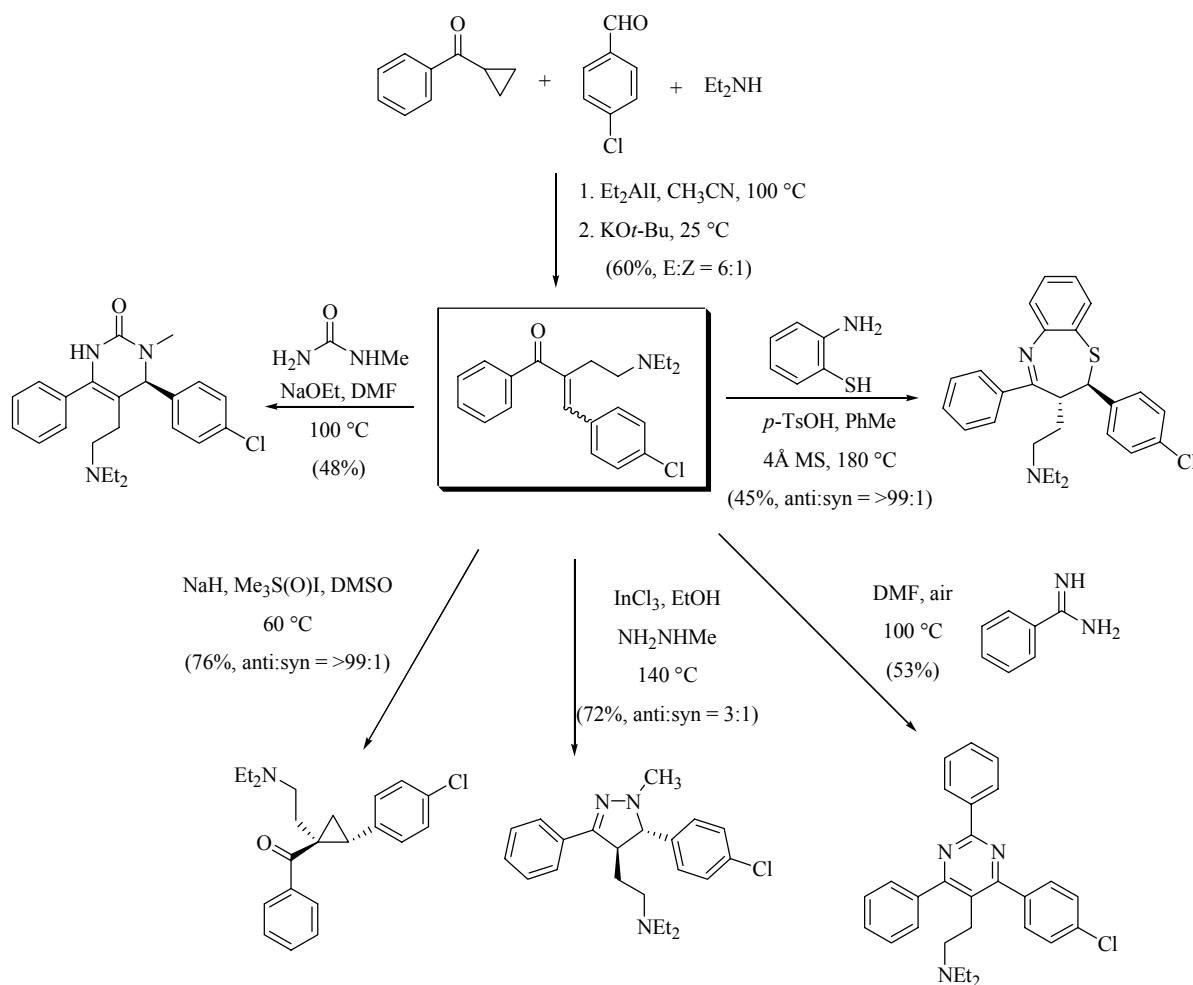
Roger Olsson, (ACADIA Pharmaceuticals A/S), Glastrup, Denmark.

Dr. Olsson presented a combinatorial scaffolding protocol aimed at the development of efficient synthesis of drug-like compounds by multicomponent reactions (MCRs). General features of these multicomponent reactions include the robust chemistries, general one-step procedures, the use of commercially available reagents, and the easy work-up procedures. A novel MgI_2 or Et_2AlI promoted three-component reaction between cyclopropyl ketones, aliphatic/aromatic aldehydes, and primary amines resulted in the formation of substituted pyrrolidines in good to excellent diastereoselectivities via the intermediate imines (Scheme 1).



Replacement of this multicomponent reaction with secondary amines would generate a pyrrolidinium salt intermediate which could undergo Hofmann elimination in the presence of base to give α -substituted α,β -enones (Scheme 2). This enone intermediate served as a “combinatorial scaffold” in the generation of drug-like heterocyclic compounds such as dihydropyrimidinone, cyclopropyl ketone, pyrazoline, pyrimidine, and benzothiazepine scaffolds. Microwave irradiation was also employed to accelerate the reaction rates compared to conventional heating.

Scheme 2



“The Role of the Chemist in Fueling the Pharmaceutical Drug Discovery Engine,”

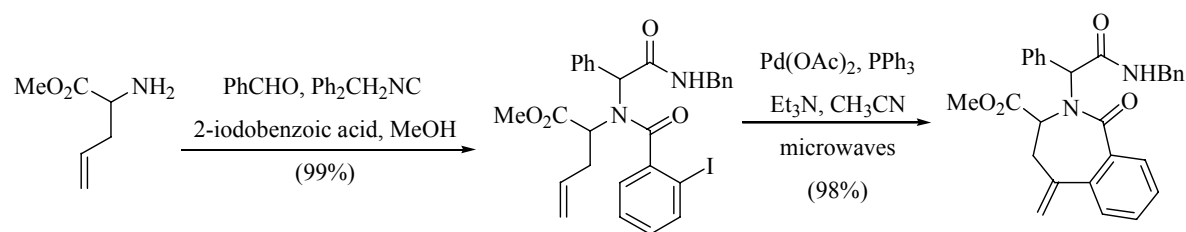
Stevan W. Djuric, (Abbott Laboratories), Abbott Park, IL.

Dr. Djuric briefly described several features of The Abbott Chemistry Engine:

1. Focused libraries for protein family targets of interest including kinases, GPCRs and ion channels.
2. Unique drug-like and lead-like pharmacophore identification through novel chemistry.
3. External collaborations to fill in missing “biologically relevant” diversity space in the Abbott compound collection.

Most of the presentation was focused on novel chemistry for drug-like and lead-like pharmacophore identifications involving sequential Ugi multicomponent coupling reactions. For example, sequential Ugi/Heck cyclization strategies for the facile construction of highly functionalized *N*-heterocyclic scaffolds such as seven-membered lactams (Scheme 3). Highly functionalized six-membered lactams were the other examples shown.

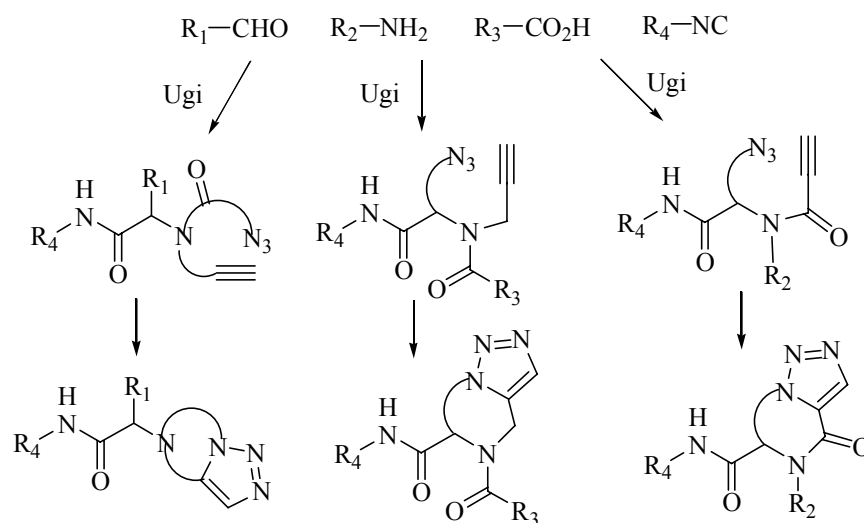
Scheme 3



Seque

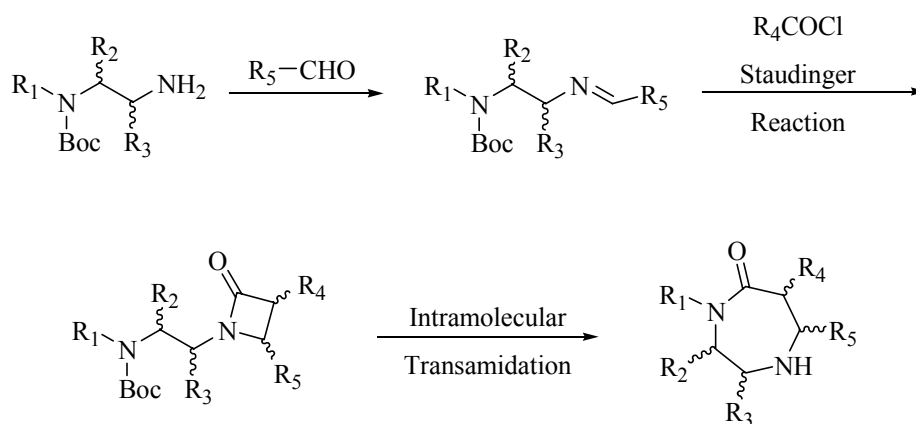
ntial Ugi/alkyne-azide cycloaddition reactions provided a versatile synthesis of fused dihydrotriazolo[1,5-*a*]pyrazinones and triazolobenzodiazepines (Scheme 4). This sequence provided access to highly functionalized heterocyclic ring systems in two steps from easily available starting materials.

Scheme 4



Diazepinones were prepared from the intramolecular transamidation ring-opening strategy of β -lactams, prepared from the Staudinger reaction of *N*-Boc-protected imines (Scheme 5). Substitution patterns of up to six positions could be varied, where four centers could be prepared stereospecifically.

Scheme 5



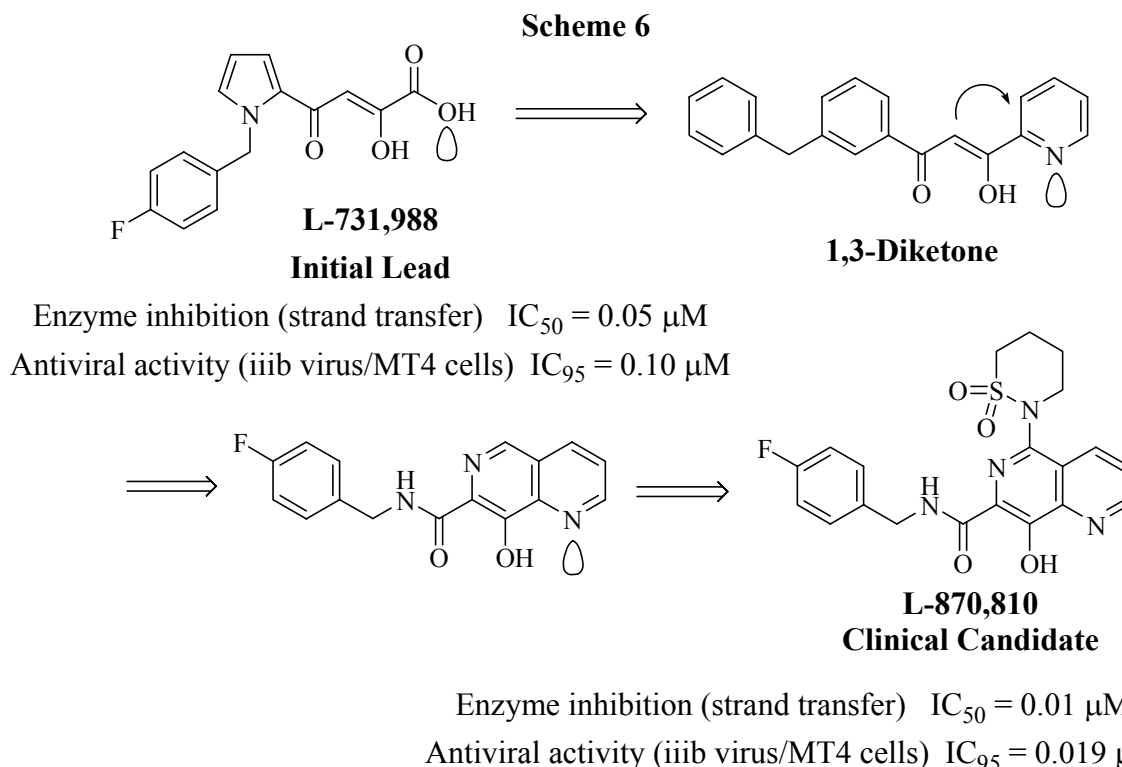
“The Discovery and Development of Potent HIV Integrase Inhibitors,”

Joe Vacca, (Merck Research Laboratories), West Point, PA.

Most current HIV therapies target two viral enzymes, HIV-1 reverse transcriptase and HIV-1 protease, to interrupt the viral replicative cycle. The HIV-1 genome encodes a third enzyme, HIV integrase, which catalyzes the integration of double-stranded viral DNA into the host cell's genomic DNA. Three biochemical steps are involved: the assembly of integrase on viral DNA, endonucleolytic cleavage of the first two nucleotides from each 3' terminal of the viral DNA, and strand transfer of the recessed viral DNA to the host cell DNA. So far no HIV integrase inhibitors are on the market for the treatment of AIDS.

Dr. Vacca outlined Merck's initial lead to a clinical candidate for a HIV integrase inhibitor program. The initial screening hit 4-aryl-2,4-diketobutanoic acid (**L-731,988**) underwent several lead optimization studies via carboxylic acid replacements with pyridyl groups (1,3-diketone) to the naphthyridine carboxamides which are bioisosteric with 2,4-diketobutanoic acids (Scheme 6). Clinical candidate **L-870,810** was found to be a potent inhibitor of HIV integrase, had good pharmacokinetics in three animal species (rat, dog, rhesus), was mechanistically distinct from currently available agents, and pharmacokinetics in humans was suitable for twice daily dosings. **L-870,810** exhibited broad-spectrum activity against wild-type and multidrug resistant HIV-1, HIV-2, and SIV strains.

Two assays were performed for each compound: Integrase strand transfer test, which measures the ability of the test compound to inhibit the integration of preprocessed donor DNA fragment into target DNA, and viral spread test, which is a three day multiple-cycle infectivity assay measuring the inhibition of viral spread with H9/iiib virus in MT4 cells to >95% as detected by p24 ELISA.

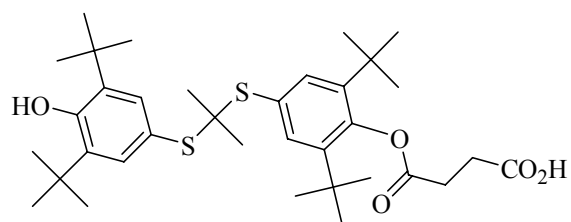


“Discovery of a New Class of Antiasthmatic Compounds,”*Charles Q. Meng, (AtheroGenics, Inc.), Atlanta, GA.*

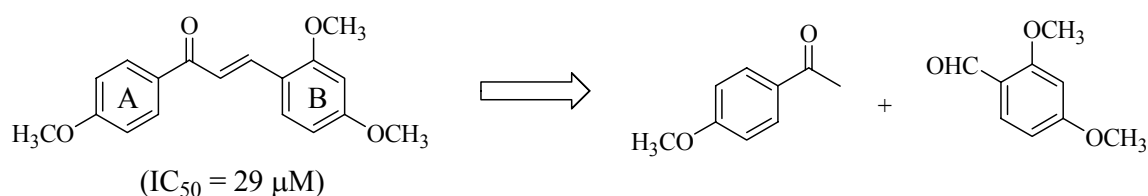
The main pathological feature of chronic inflammatory diseases is infiltration of the affected area by leukocytes. Vascular cell adhesion molecule-1 (VCAM-1), which is induced by mediators such as TNF- α or IL-1, plays an important role in this process by binding to its receptor on leukocytes. Inhibition of VCAM-1 expression has shown therapeutic efficacy in various animal models of chronic inflammation, and thus can be considered as a promising therapeutic approach for complex inflammatory diseases.

Asthma, the chronic inflammatory disease of the airways, affects about 10% of the population. VCAM-1 has been identified as a promising asthma marker since VCAM-1 is unregulated in the airways of healthy individuals following an antigen challenge of asthmatic patients. In addition, VCAM-1 antibodies have been effective in asthma models of different species.

Dr. Meng described the development of a novel inhibitor of VCAM-1 expression. After developing **AGI-1067**, a first-generation inhibitor of VCAM-1 expression currently in phase III for atherosclerosis, a screening for a new series of inhibitors was conducted (Figure 1).

Figure 1**AGI-1067** $(IC_{50} = 6 \mu M)$

Some natural products have been reported in the literature as potent inhibitors of VCAM-1 expression. A common structural feature of these natural products is an α,β -unsaturated carbonyl, which prompted AtheroGenics to screen compounds containing this moiety for inhibition of VCAM-1 expression and this led to the discovery of a chalcone as a new structural lead. The chalcone lead could easily be obtained from the reaction of an acetophenone and benzaldehyde in the presence of base (Scheme 7).

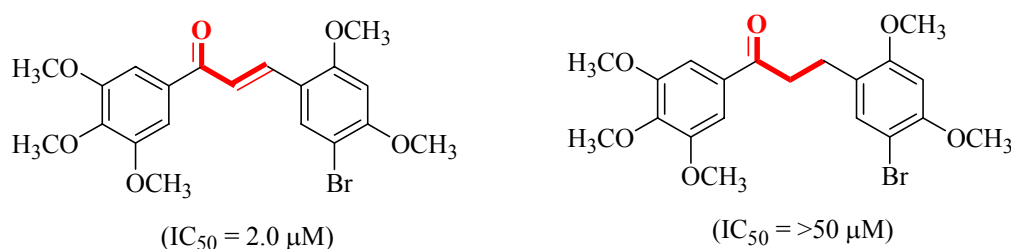
Scheme 7

Various chalcone derivatives were screened against VCAM-1 expression. SAR studies of the initial chalcone compounds indicated that methoxy and thienyl substituents on ring B helped boost inhibitory potency on VCAM-1 expression. However the initial compounds were highly insoluble in water. Introduction of a carboxy group onto ring A of the chalcone improved this aspect without affecting biological activity.

Summary of the SAR studies:

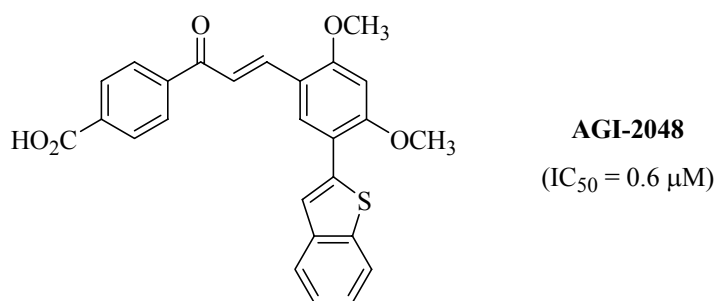
- Heteroaryl substitutions on ring B helped boost potency; *meta*-substitution was optimal.
- Potency decreased dramatically when both 2- and 6-position on ring B were occupied.
- Certain range of lipophilicity was essential for optimal *in vitro* potency besides the requirements of special configuration of compounds.
- The α,β -unsaturated carbonyls were essential (Figure 2).

Figure 2



AGI-2048 (Figure 3) from the SAR-study was then selected to undergo further biological studies based on its favorable *in vitro* potency, scalability and formulability. **AGI-2048** demonstrated *in vivo* proof-of concept for asthma by dose-dependency inhibiting eosinophilia in a murine model of allergic asthma. IgE levels were lowered and the lung functions were improved.

Figure 3

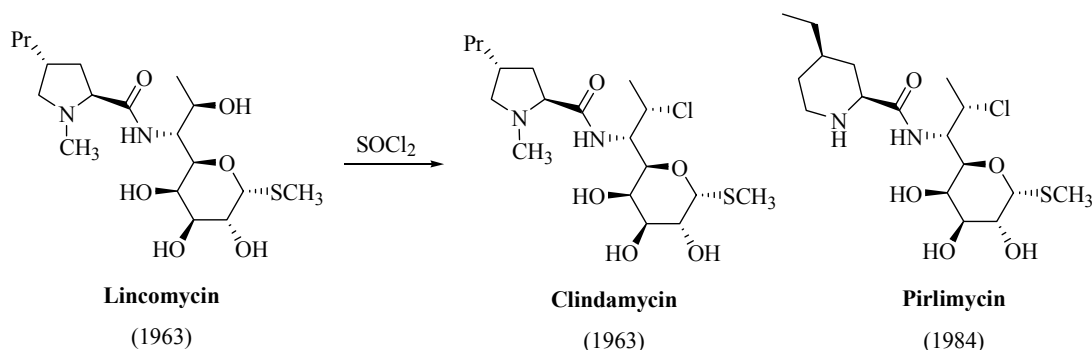


“Novel Synthesis of Lincosamides and Discovery of Antibacterial Agent VIC-105555,”
Jason G. Lewis, (Vicuron Pharmaceuticals), King of Prussia, PA.

Lincosamides act by interfering with the process of peptide elongation in bacterial protein synthesis with a similar binding site to the group of macrolide antibiotics to which erythromycin belongs. These antibiotics are active against gram-positive cocci and against many anaerobes

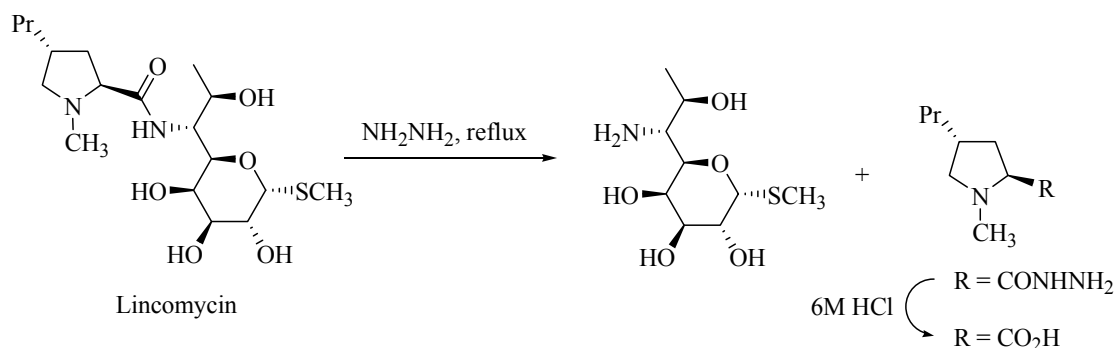
(including Bacteriodes). Lincomycin and Clindamycin are two of the antibiotics of the lincosamides class. Lincomycin was first isolated beginning of the 1960s from the microorganism *Streptomyces lincolnensis* (Figure 4). Clindamycin, which was developed from lincomycin, had largely replaced lincomycin because of better absorption and activity. Because of serious side-effects clindamycin and lincomycin have only a few specific indications. To develop lincosamides with improved pharmacokinetic properties and antibacterial activities, a simple entry to modifications of the complex structure of these compounds was required.

Figure 4



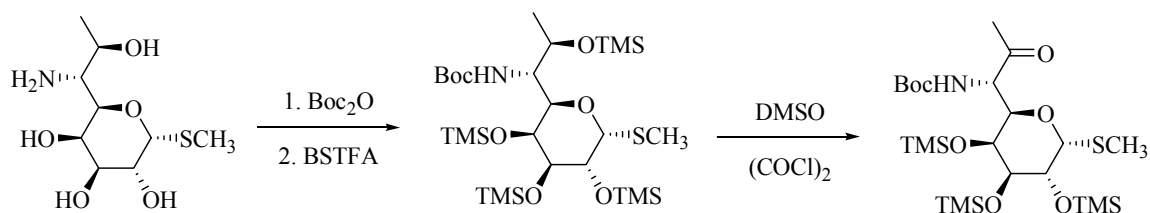
Dr. Lewis described a new methodology of a selective oxidation of the 7-position of sugar moiety. In a first step, the molecule was cut into two pieces: Hydrazinolysis of lincomycin generated the amine and the hydrazide which was hydrolyzed with 6M HCl to give the acid (Scheme 8), which was used later in the SAR studies.

Scheme 8



Boc-protection of the amine and subsequent hydroxyl group protections generated the silyl-protected intermediate, which underwent Swern oxidation selectively at the 7-position to give the ketone (Scheme 9).

Scheme 9



The key ketone intermediate allowed entry to several C-7 modified analogs such as oximes and C-alkylated compounds (Scheme 10). Amide coupling of the amines with the acid (from Scheme 8) or pipercolinic acid derivatives led to SAR studies to identify **VIC-105555**, a structurally novel lincosamide analog with an improved antibacterial spectrum, pharmacokinetics, and potency, combined with a low frequency of *in vitro* spontaneous resistance (Figure 5).

Scheme 10

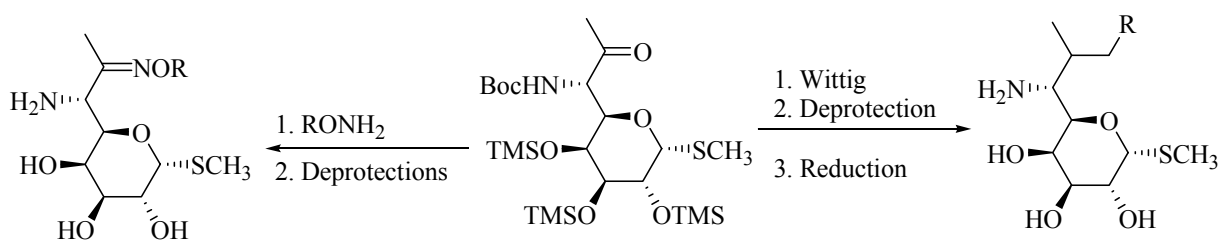
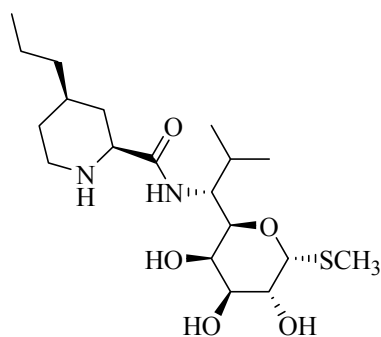


Figure 5



VIC-105555

“Synthetic Library Design,”

Christoph Huw, (Schering AG), Berlin, Germany.

The shift from drug discovery methods based on traditional combinatorial chemistry and large compound collections to the use of focused libraries designed around biological targets has improved the pace and productivity of discovery and medicinal chemistry programs.

Dr. Huw described briefly why and how synthetic libraries should be designed. The rational reason for the design of libraries is that, although in our days easily a very large number of chemical compounds are accessible via automation and combinatorial techniques, quality standards (purification) and the limitations of resources (capacity, timelines) require an initial

selection of compounds. Requirements of an appropriate potency, selectivity, solubility, oral bioavailability, etc. can be addressed by an additional step of refinement.

Such a combination of a target- and structure-based library design followed by a property-biased library refinement has proven to be useful and some examples were given:

- Target- and structure-based library design: The combination of computational and combinatorial chemistry to guide synthetic efforts.
- Property-biased library refinement: *In silico* property scoring (Customized Rule-of-Five, druglikeness versus leadlikeness, dynamic and static polar surface area).
- Final selection by medicinal chemist based on know-how of medicinal chemistry, and structure-activity-relationship considerations.

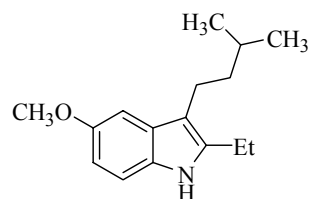
“A Conceptual Framework Model for the Design of New and Selective 5-HT₆ Ligands,”

Jorg Holemz, (Esteve Laboratories, Departments of Medical Chemistry and Discovery Biology), Barcelona, Spain.

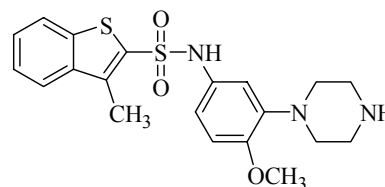
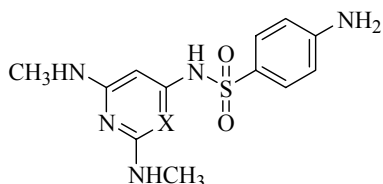
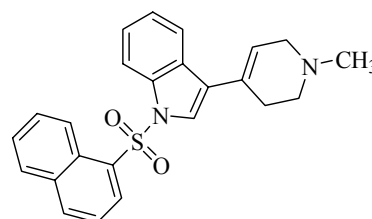
Over the past 5 years, serotonergic agents have been the major focus for CNS disease as exemplified by 90% of the patent applications. There are 7 major classes of serotonin receptors (5-HT₁₋₇) which are expressed in the mammalian CNS. The rat and mouse 5-HT₆ receptors were described in 1993 and 1994, respectively, and the human 5-HT₆ receptor was first reported in 1996, being positively coupled to adenylyl cyclase.

5-HT₆ serotonin receptors are members of the G-protein family. The 5-HT₆ receptor appears to regulate several neurotransmitter systems. Its unique distribution in the brain and high affinity for therapeutic antipsychotics and antidepressants suggests a role of the receptor in CNS disorders. The importance of the 5-HT₆ receptors in memory and learning has also been postulated in rats. Recently, the synthesis of a series of novel ligands have been reported which introduced a variety of novel compounds as potent and selective binders for the 5-HT₆ receptor. The first selective 5-HT₆ agonist was 2-ethyl-5-methoxy-*N,N*-dimethyltryptamine (**EMDT**). Selective 5-HT₆ antagonists reported include benzenesulfonamides **Ro 04-6790** and **Ro 63-0563**, the benzene[*b*]thiosulfonamide **SB-271046** and the sulfonylindole **ALX1175** (Figure 6).

Figure 6



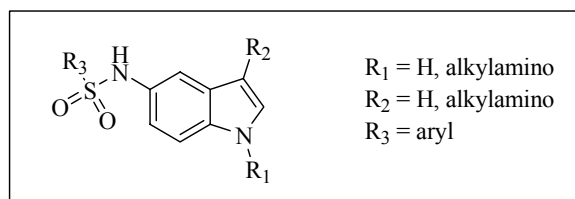
EMDT (agonist)

SB-271046, $K_i = 0.8$ nMX = N (**Ro-04-6790**), $K_i = 44.7$ nMX = CH (**Ro-63-0563**), $K_i = 14.6$ nM

ALX1175

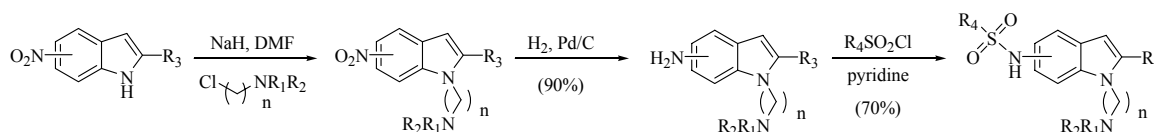
In order to further study the 5-HT₆ receptor and its physiological function, different series of indolyl sulfonamides were prepared based on a medicinal chemistry guided conceptual framework model. These potent ligands were investigated in terms of their structure-affinity relationships (SAFIR). The structural requirements within this framework comprise of two hydrophobic regions separated by a double electron receptor functional group (commonly a sulfonamide) and a proton donor (represented by an amino group that is protonated at physiological pH). These features were applied to the core structure below (Figure 7).

Figure 7

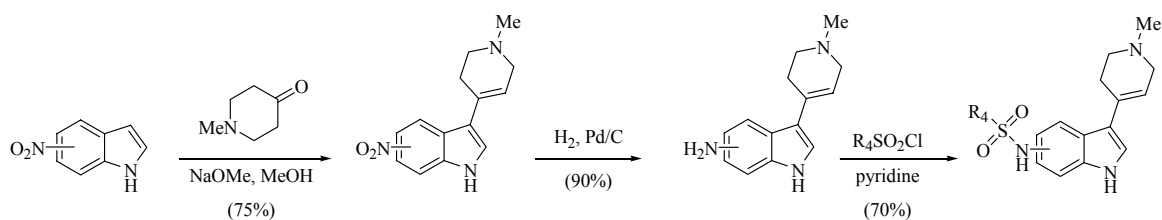


Novel series of indolyl sulfonamides with different substitution patterns were prepared as potential 5-HT₆ ligands. These were grouped into four classes based on their common scaffolds as shown in Schemes 11-13.

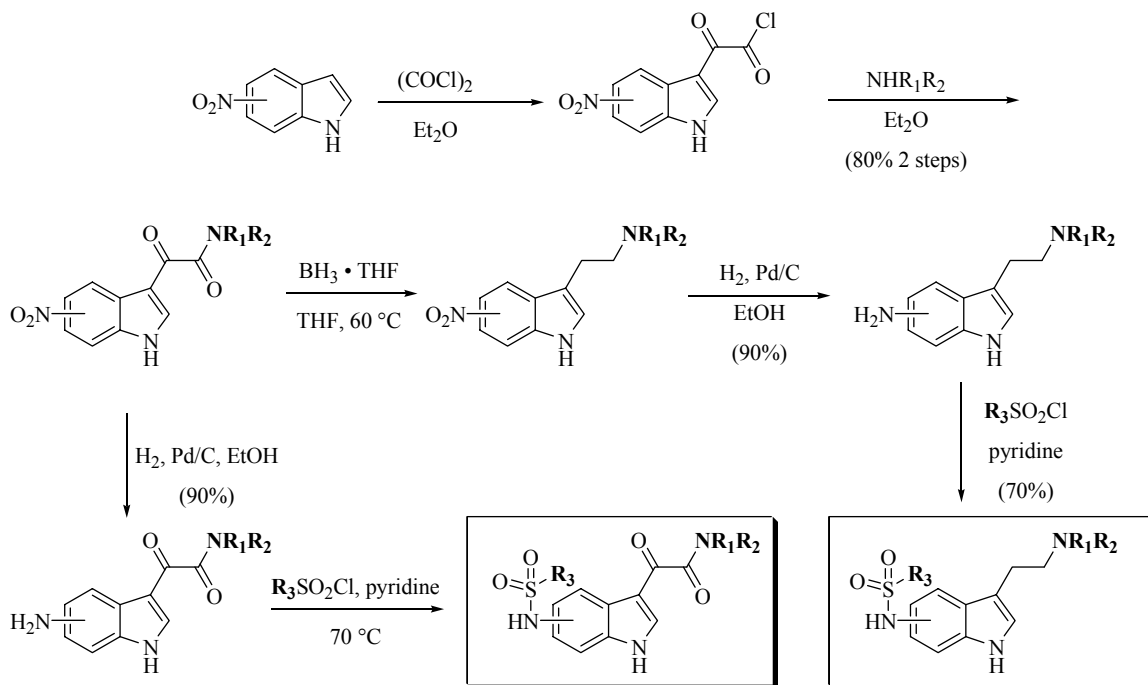
Scheme 11



Scheme 12

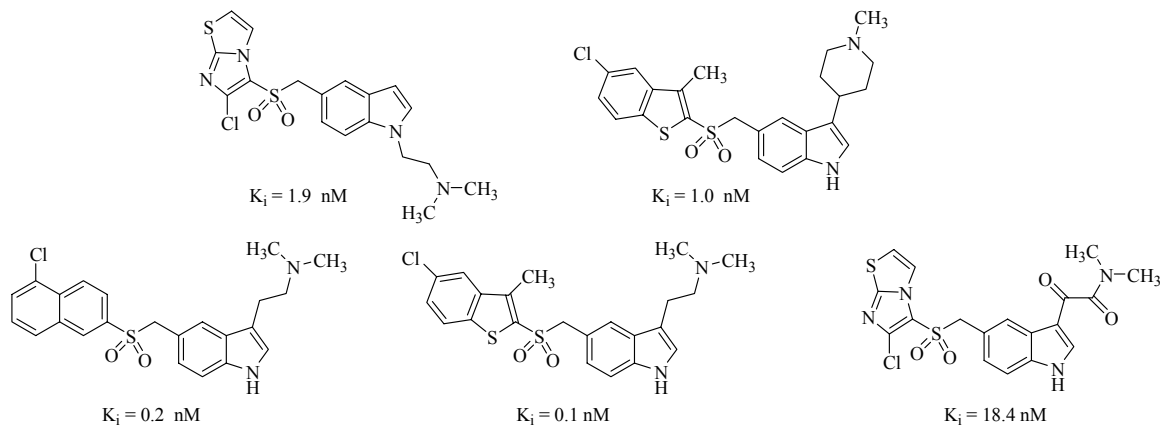


Scheme 13



Examples of potent 5-HT₆ ligands prepared in this manner are shown below (Figure 8):

Figure 8



This approach led to the development of novel and highly potent 5-HT₆ ligands with defined functionalities. These targets were selective with respect to >75 other receptors and were bioavailably and metabolically stable. *In vivo* studies have indicated promising effects in cognitive enhancement and obesity trials. Currently, further candidate profiling is ongoing.

“Training Medicinal Chemists: Making the Transition,”

Paige E. Mahaney, (Wyeth Research), Collegeville, PA.

This presentation highlighted the current industrial trend in the drug discovery industry of short timelines and increase productivity. There is a heavy investment in high throughput technologies; R&D metrics are used to measure productivity, innovation and business growth to ensure success in changing the pharmaceutical environment. Consequently, new industrial scientists must be rapidly assimilated into this environment.

The role of chemistry comprise of many specialist functions across the drug discovery process from target identification through to candidate selection. Moreover, when recruiting from academia, pharmaceutical companies look for knowledge in organic chemistry and expertise in synthetic organic chemistry. It is also a common view that an accomplished medicinal chemist is a skilled synthetic organic chemist who finally learned some biology. The task of training medicinal chemists is an arduous one given that in academia there are not many chemistry departments which prepare students for careers in drug discovery by introducing them to modern drug design concepts.

For new hires, there are a number or residential short courses in medicinal chemistry available, but these are often oversubscribed. To overcome this problem, Wyeth provided a mentoring system where new hires report to a senior (non-manager) scientist.

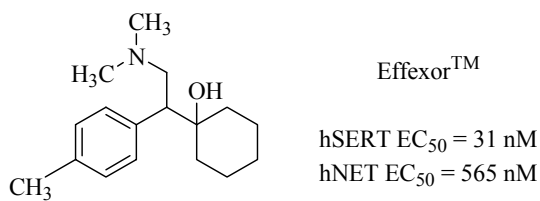
The supervisor's responsibilities include:

- Instruction in Wyeth infrastructure (corporate databases, etc.)
- Instruction in medicinal chemistry, physical organic chemistry, pharmacology, modeling, etc.

After a period of time, supervisors may change.

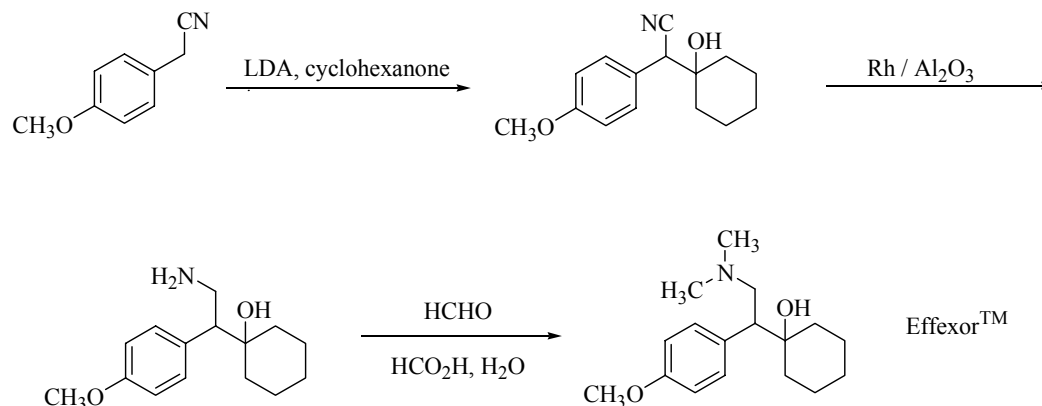
Case studies were also used as part of this training. One example discussed involved the parallel synthesis of effexor analogs (Figure 9).

Figure 9



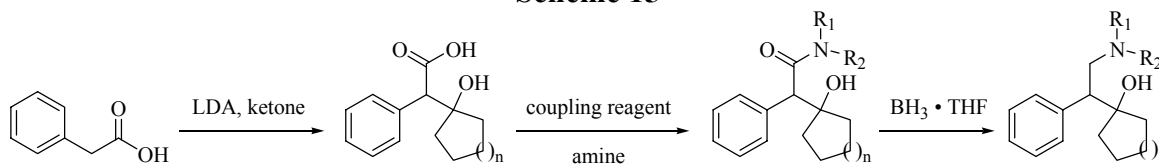
The original synthesis started with (4-methoxyphenyl)acetonitrile which was deprotonated with LDA and treated with cyclohexanone (Scheme 14). Reduction of the nitrile group with rhodium on alumina followed by reductive amination with formaldehyde gave effexor.

Scheme 14



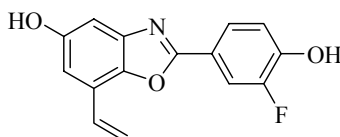
This approach was modified to provide a matrix approach for analog synthesis which led to the synthesis of 27 matrix analogs (Scheme 15).

Scheme 15



A second case study was presented on structure-based drug design and molecular modeling which was used for the search of ER- β selective agonists for the treatment of inflammatory diseases. Multiple X-ray co-crystal structures of modestly selective ligands complexed to both ER α and ER β , along with docking calculations were used to take advantage of a single conservative residue substitution in the ligand binding pocket to optimize ER β selectivity. This approach led to the discovery of compounds such as **ER β -041**, a >200-fold ER β selective agonist (Figure 10).

Figure 10



WAY-202041
(ER β -041)

IC₅₀ = 5 nM (ER β)

(Selectivity 226-fold)

“Adventures in Diversity Oriented Synthesis,”

David R. Spring, (Department of Chemistry, Cambridge University), UK.

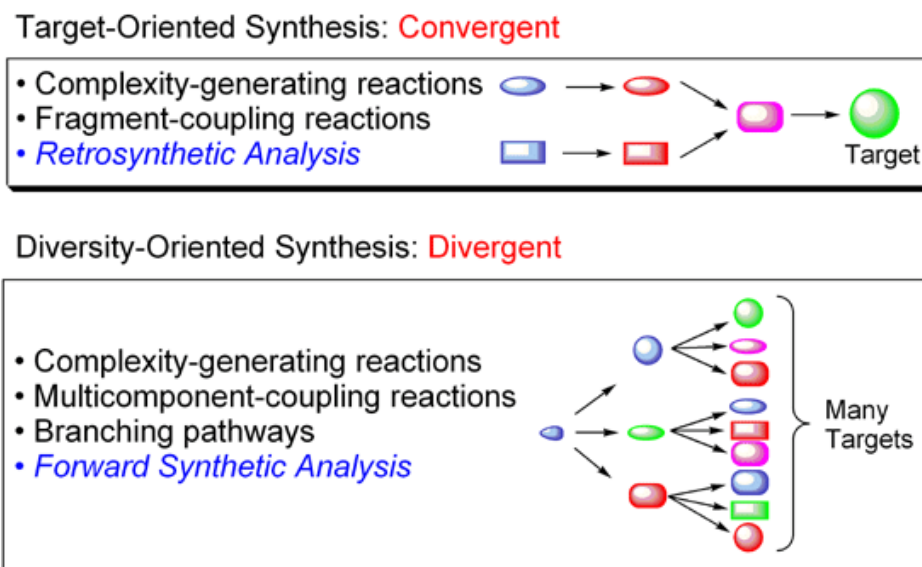
The development of new antibacterial agents has received relatively little investment by major pharmaceutical and biotech companies. Due to multidrug-resistant bacterial pathogens, infectious disease mortality in the developed countries is increasing. In order to address this problem, Professor Spring has adopted a chemical genetic approach to discover new antibacterial modes of action. This involves the study of biological processes using small molecule (‘chemical’) intervention, instead of genetic intervention. The main disadvantage of small molecules is that there is no general approach. Furthermore, there is a need for a small molecule modulator for each function of all gene products: chemical genomics. The number of possible ‘drug-like’ molecules has been calculated to be astronomical.

In order to set about this task, several criteria must be considered:

- Quality and, but not just, quantity are important.
- Structurally similar compounds have similar biological activities.
- There is more than one answer to every (biological) problem.

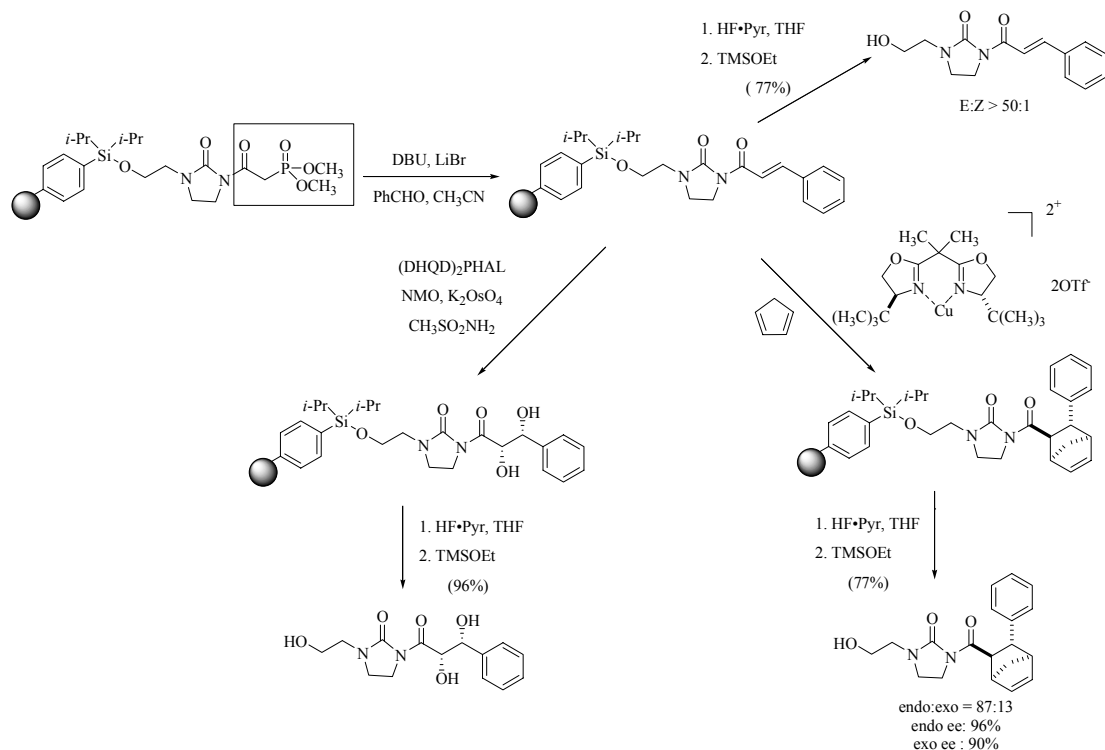
These issues can be addressed by the synthesis of structurally diverse small molecule collections. Consequently, Professor Spring has applied a Diversity Oriented Synthesis (DOS) approach to this complex problem. DOS is defined as the deliberate, simultaneous and efficient synthesis of more than one target compound (Figure 11). This is in contrast to Target Oriented Synthesis (TOS). DOS proceeds via a forward synthetic analysis which reveals that the key to achieving structural diversity is inventing branching reaction pathways, where a common substrate is used in different reactions that give different atomic frameworks. It also reveals that the key to efficient generation of structural complexity is the adoption of complexity generating reactions and multi-component coupling reactions.

Figure 11



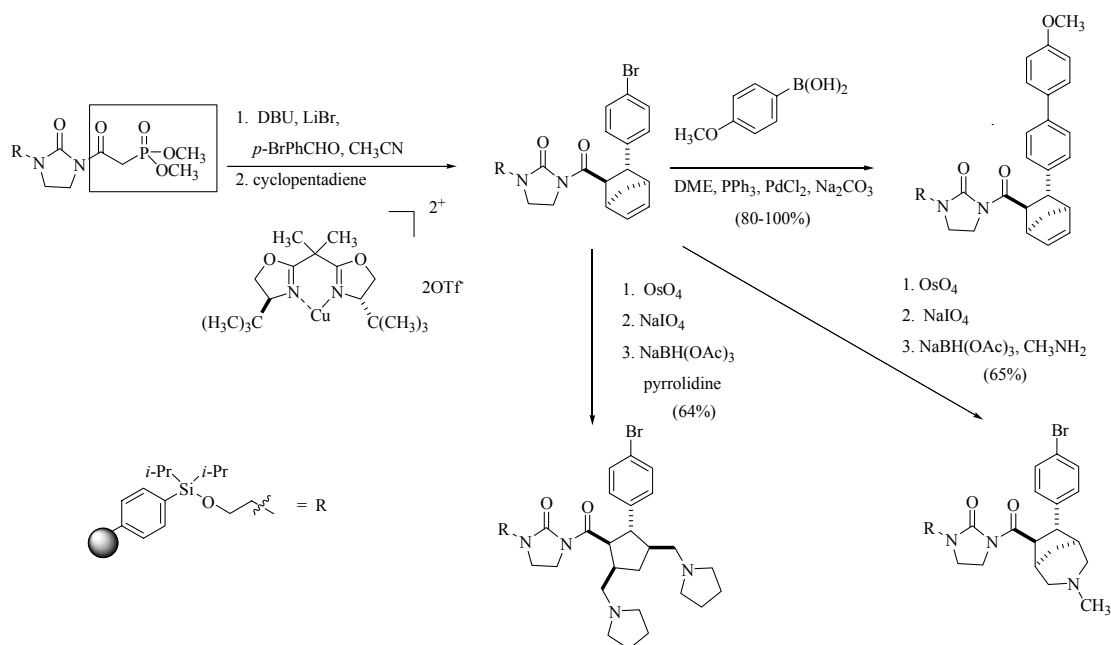
One example presented which illustrated this concept is shown in Scheme 16. Wittig olefination of immobilized ketophosphonate with benzaldehyde gave the *trans* cinnamyl olefin which could undergo either asymmetric dihydroxylation or asymmetric Diels-Alder cyclization.

Scheme 16



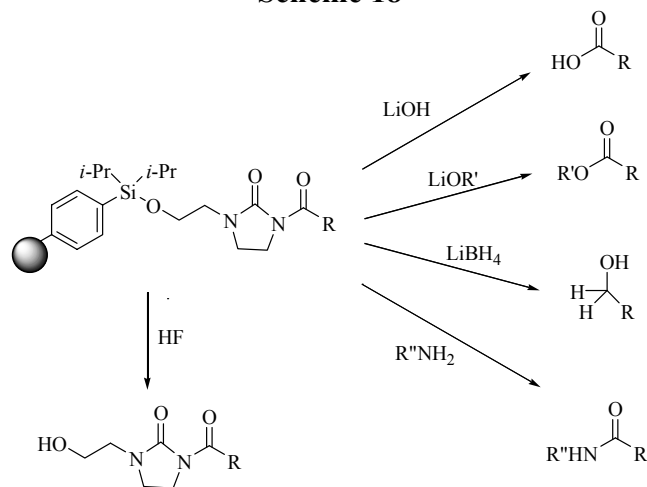
Wittig olefination of ketophosphonate with 4-bromobenzaldehyde followed by asymmetric Diels-Alder reaction with cyclopentadiene provided a cyclic intermediate which could undergo further Suzuki coupling to give a cross-coupled product (Scheme 17). Furthermore, the norbornene ring of the cyclic intermediate could be oxidatively cleaved to give the intermediate bisaldehyde, which could undergo reductive amination with pyrrolidine or with methyl amine.

Scheme 17



Alternatively, the immobilized tether can be cleaved at the silicon oxygen bond or the amide bond which could lead to a number of diverse products depending on the method used (Scheme 18).

Scheme 18



“Kinase Profiling and the Specificity of Kinase Inhibitors,”

David Lockhart, (Ambit Biosciences), San Diego, CA.

Protein kinases are important targets for drug development, with over 30 kinase inhibitors currently in clinical development, and many more in preclinical assessment. They play important roles in signal transduction in cells, and are targeted for the treatment of many diseases, including cancer, CNS indications, metabolic disorders and inflammation. Most kinase inhibitors target the kinase ATP site common to all protein kinases. Therefore, assessment of inhibitor specificity is crucial to identify off-target interactions. However, molecular specificity is not readily predictable (based on available structural information). The measurement of kinase inhibitor specificity

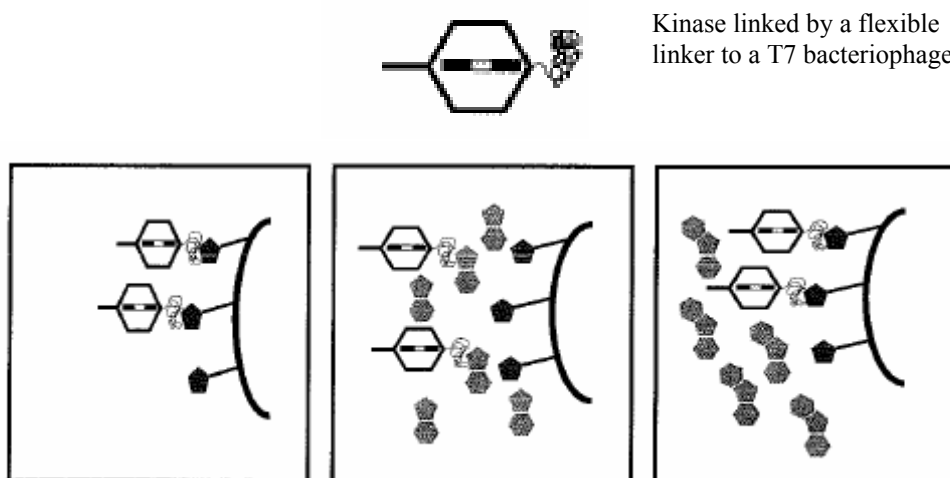
requires the assessment of a given inhibitor versus a large panel of kinases and kinase mutants. Scientists at Ambit Biosciences have devised an experimental approach toward assessing the specificity of kinase inhibitors.

The key components of Ambit's assay are full-length, folded human kinases tethered via a flexible linker to a proprietary T7 phage particle (Figure 12). The T7 phage allows for the amplification of the attached protein. Kinases are cloned into the phage vector, which are grown in *E. coli*. Phage replication leads to the lysis of the bacteria, and the lysates are directly used in the assay, thus obviating the need for conventional protein purification. The assay also uses a small set of immobilized ligands ('bait'); nine different 'bait' molecules are used, and were selected for their ability to bind to the ATP sites of several kinases with high affinity. The 'bait' ligands were attached to biotin via a flexible chemical linker; biotinylated ligands were then immobilized on streptavidin-coated beads. 'Free' test compound is added to the tagged kinases and the immobilized 'bait'; if the test compound binds to the tagged kinase, then less protein is bound to the 'bait' ligand, and vice versa. The amount of kinase bound to the immobilized ligand in the presence and absence of 'free' test compound is measured by quantifying the amount of protein bound to the solid support by quantitative PCR or phage plaque assays. The sensitivity of these measurements allow for the detection of as few as 10-100 tagged protein molecules.

The main advantages of the assay are as follows:

1. The assay quantitatively measures binding to the ATP site.
2. It is a competitive binding assay, highly correlated to with effects on enzyme activity.
3. It does not require chemical linking, labeling or immobilization of test compounds.
4. Compounds are profiled against an entire panel of kinases in parallel (>180); this allows for rapid and quantitative K_d determination.
5. Conventional protein expression/purification is not required.
6. No 2D-gel electrophoresis or mass spectrometry is required.
7. No kinase substrate is needed.
8. Results are not dependent on ATP concentration (no ATP added).
9. No need to individually express, purify and stock each kinase of interest.

Figure 12: Schematic of a tagged protein, and competition binding assay



No test compound**Test Compound
Competition No Competition**

As of April 2005, 156 distinct kinases were represented in Ambit's high-throughput profiling panel (180, if mutant forms were included). Assay validation was carried out by comparing K_d 's obtained with published IC_{50} or K_i values; good correlation between binding constants measured by this assay and published IC_{50} values was observed.

Ambit scientists have used the competition assay ('Kinomescan') to profile the specificity of twenty known kinase inhibitors. These compounds were structurally diverse, and inhibited a variety of kinases. Their results showed that specificity varied widely among this set of inhibitors. For example, staurosporine, a molecule known to be a highly promiscuous inhibitor, inhibited a majority of the kinases in the panel (the binding affinity varied between 20 pM to 7 μ M). Others, such as SU-11248, Vatanalib, BIRB-796 and VX-745, exhibited at least a 10-fold selectivity between targeted and off-target kinases. Even compounds based on the same chemical scaffold exhibited widely differing specificities, e.g., Iressa, Tarceva and GW-2016 (all EGFR inhibitors based on quinazoline scaffolds). Of the three, GW-2016 is the most specific, while Iressa and Tarceva bind more off-target kinases than GW-2016.

Scientists at Ambit have used this proprietary technology to develop a series of FLT3 inhibitors. FLT3 is a target for acute myeloid leukemia (AML); patients with activating mutations in FLT3 are less likely to respond to conventional treatment. Data presented for this series of compounds revealed that they had high affinity, specificity and potency in cell-based assays, as well as good kinase specificity profiles. About 20% of the candidate compounds had good oral bioavailability. Results from the safety assessment of these compounds (hERG and Cyp isoforms) are also promising. All new Ambit compounds are screened against a panel of 'high value' kinases, including FLT3, KIT (wt and mutant), ABL (wt and mutant), Aurora A and VEGFR2. An example that was presented concerned Gleevec-resistant mutated versions of the ABL kinase, implicated in chronic myeloid leukemia (CML). These mutations decrease sensitivity to Gleevec. Thus, second-generation drugs which inhibit these mutant ABL kinases are necessary to treat CML in patients who have developed resistance to Gleevec. A number of known kinase inhibitors were screened for their ability to bind to a set of six most commonly observed ABL kinase mutants. Of these mutant ABL forms, one, T315I, is almost completely resistant to Gleevec (as well as other known kinase inhibitors and ABL kinase inhibitors). The data revealed that both BIRB-796 (a p38 inhibitor) and VX-680 (an Aurora kinase inhibitor) bind to the T315I ABL mutant. Furthermore, one of Ambit's own compounds, AB200515, binds this mutant as well. Both BIRB-796 and AB200515 inhibit ABL (T315I) in a cell proliferation assay. Ambit is currently working on constructing a focused library base on AB200515 to find additional ABL (T315I) inhibitors. Ambit scientists are also working on Aurora A kinase inhibitors (in the examples given was Ambit's AB500038, $K_d \sim 20$ nM).

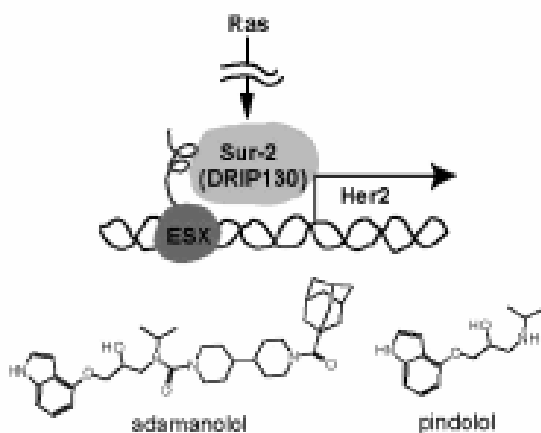
"Modulation of Gene Expression by Targeting a Protein-Protein Interaction,"

Professor Motonari Uesugi, (Baylor College of Medicine), Houston, TX.

Professor Uesugi's talk focused on the induction of drastic phenotype changes by regulation of gene transcription. Regulation of transcription by small organic molecules is a challenging goal in chemistry, as protein-protein interactions usually involve large surfaces devoid of binding pockets for small molecules. However, protein-protein interactions mediated by α -helical segments have been shown to be amenable to modulation by small nonpeptidic molecules. One such interaction exists between the activation domain of the ESX transcription factor and its co-activator Sur-2.

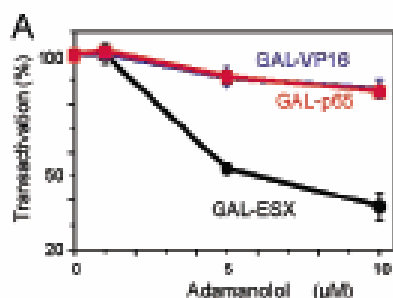
ESX is a transcription factor that activates the *Her-2* gene, which is overexpressed in ~30% of breast cancer patients (~60000 *Her-2*-positive cases per year in the US). ESX binds and activates the *Her-2* promoter (Figure 13). Also, the ESX-binding site is essential for the high-level expression of *Her-2* in breast cancer cells. Sur-2 is a co-activator of ESX, which binds specifically to the activation domain of ESX. Disruption of the ESX-Sur2 interaction may therefore constitute a viable approach to breast cancer treatment. The interaction between Sur-2 and ESX involves one face of an 8-amino acid α -helical region in the ESX activation domain (Ser-Trp-Ile-Ile-Glu-Leu-Leu-Glu), with the Trp residue being essential for the specificity of the interaction.

Figure 13: ESX-Sur-2 interaction and inhibitors of the interaction



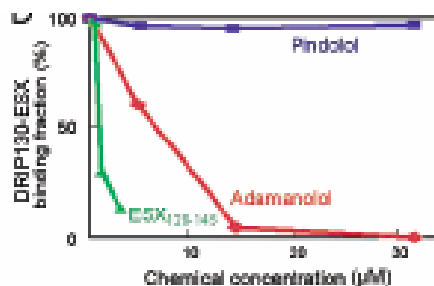
A library enriched in indole and indole-mimicking pharmacophores from Tripos' compound collection was screened in cell-based assays and led to the identification of adamanolol. Adamanolol was shown to block the ability of ESX to activate transcription of a reporter gene in cells; however, it did not affect the activation ability of two structurally similar activation domains VP16 and p65 (Figure 14). The cellular activity of

Figure 14:
Adamanolol selectively blocks activation of transcription by the ESX activation domain



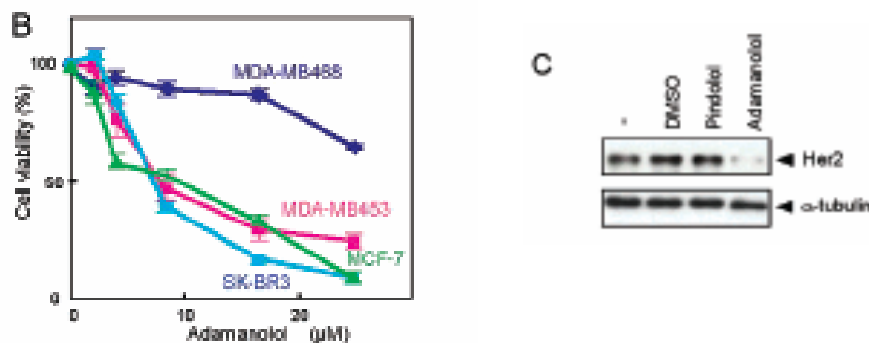
adamanolol is similar to that observed for a peptide inhibitor derived from the ESX activation domain (called ESX₁₂₉₋₁₄₅). In previous experiments, this short, α -helical peptide had been shown to be sufficient to interact with Sur-2, therefore, implying that adamanolol exerts its effect by blocking the interaction between Sur-2 and ESX. Competition experiments using FITC-labeled ESX₁₂₉₋₁₄₅ and adamanolol demonstrated that the latter inhibited the ESX-Sur-2 interaction in a dose-dependent manner (Figure 15). Pindolol, a truncated version of adamanolol, was ineffective even at concentrations of 30 μ M.

Figure 15: Adamanolol blocks the interaction of ESX with Sur-2 *in vitro*



Furthermore, adamanolol was shown to reduce Her2 expression, and also to decrease the viability of Her2-positive breast cancer cells but not Her2-negative cell lines (Figure 16). Adamanolol is thought to inhibit the ESX-Sur-2 interactions by adopting a *cis* conformation, which may mimic the α -helical interface of the ESX activation domain.

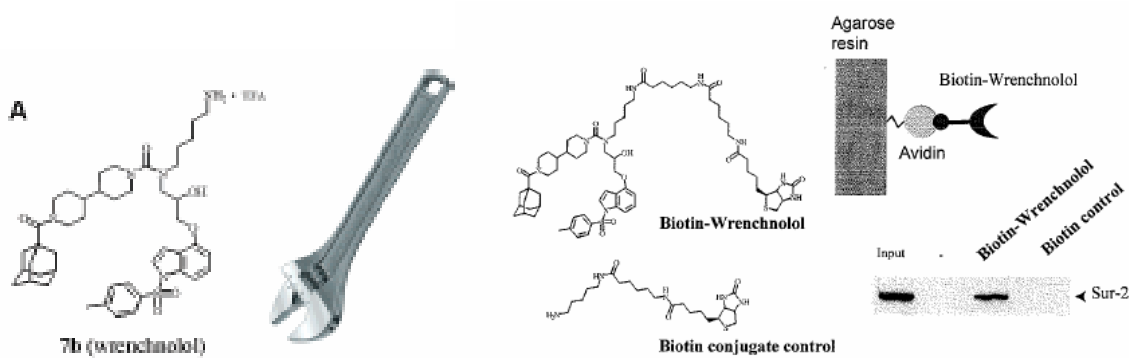
**Figure 16:
Adamanolol reduces Her-2 expression, and kills Her-2-positive breast cancer cells (MDA-MB453, MCF-7, SK-BR3), but not Her-2-negative cell lines (MDA-MB468)**



Derivatives of adamanolol wherein the bulky adamantane group was replaced by smaller groups proved to be less active than the parent compound. However, introduction of an arylsulfonyl group at the N1 position of the indole ring resulted in compounds with improved activity, as did derivatives in which the isopropyl group in the adamanolol urea region was replaced by bulky substituents. Bulky substituents in the urea portion of the molecule are thought to enforce the *cis* conformation, which may bring the indole and adamantane groups into close proximity to form the non-polar interface for interaction with Sur-2.

A second-generation compound, dubbed ‘wrenchnolol’ inhibited the ESX-Sur-2 interaction more strongly than adamanolol itself, and was also shown to be as active as the first-generation compound at killing Her-2-positive cells (Figure 17). Wrenchnolol has the added advantage of being more readily modifiable. NMR experiments revealed that the adamantane and indole groups are located in close proximity in water. Molecular modeling supports the wrench-like shape of the molecule, with the polar arm pointing away from the hydrophobic surface. Animal studies with wrenchnolol showed reduced tumor volumes in animals treated with wrenchnolol. A wrenchnolol-biotin conjugate was used to establish the selective interaction of wrenchnolol with Sur-2. Furthermore, a wrenchnolol-fluorescein was used to confirm that wrenchnolol crossed the cell membrane in breast cancer cells, and was found to be present in both the cytoplasm and the nucleus.

Figure 17: Wrenchnolol, and Wrenchnolol-Sur-2 interaction *in vitro*



In the final part of the talk, Professor Uesugi described the design of a synthetic transcription factor comprising of a wrenchnolol-hairpin polyamide conjugate (called synthetic transcription factor or STF1). The hairpin polyamide portion functions as the DNA-binding module, while wrenchnolol functions as the activation module. *In vitro* experiments revealed that STF1 can activate transcription. Further efforts are currently underway to improve the cell permeability of STF1.

“Novel PKC Agonist Design,”

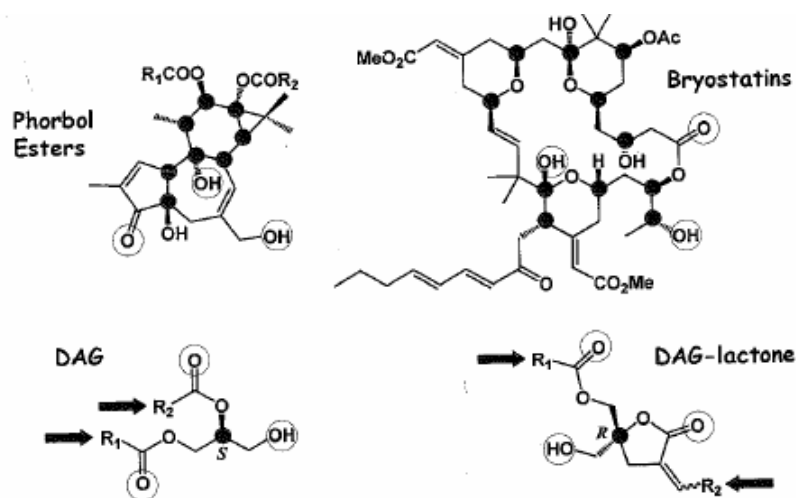
Victor E. Marquez, Ph.D., (National Cancer Institute – Frederick, NIH), Frederick, MD.

Protein kinase C (PKC) plays a central role in signal transduction and is an important therapeutic target for cancer. Diacylglycerol (DAG) is a secondary messenger that interacts with proteins containing a C1-domain, leading to their activation or translocation to different cellular sites. DAG is generated by a variety of pathways; consequently, the cell can, via differential modulation of DAG, generate a wide variety of responses. The PKC isozymes constitute important DAG receptors, and comprise eleven family members, divided into 3 subclasses: classical (cPKCs), novel (nPKCs) and atypical (aPKCs). cPKCs and nPKCs both bind DAG, while aPKCs are insensitive to DAG. All three classes contain a C-terminal kinase domain (Ser/Thr specific), and an N-terminal regulatory domain. The regulatory domain contains a pseudosubstrate, which is thought to occupy the catalytic site. Multiple interactions at the regulatory domain lead to the kinase adopting an unfolded configuration, resulting in the release of the pseudosubstrate from the

active site, and enzyme activation. Other regulators of PKC activity include calcium, as well as the cellular membrane.

Known active site inhibitors of PKC include analogues of the natural product staurosporine. The Marquez group decided to focus on a second approach to the inhibition of PKCs (given the widespread occurrence of protein kinases, as well as the homology in their active sites), namely, the modulation of the C1-domain. Support for this second approach may be garnered from nature; numerous natural products, such as the phorbol esters, bryostatins, polyacetates such as aplysiotoxin, and indole alkaloids such as teleocidin are PKC ligands, and elicit a wide variety of biological responses (Figure 18).

Figure 18: PKC Ligands



Furthermore, PKC family members may be mutually antagonistic, i.e., some isoforms are upregulated, while others are downregulated in different kinds of cancer, e.g., PKC α and PKC β are overexpressed in breast cancer cells, while PKC δ and PKC η are downregulated (Figure 19). The Marquez group hopes to exploit this mutual antagonism of isoforms to arrive at useful therapeutics for the treatment of cancer and other diseases. Their research is focused on deriving these therapeutics by appropriate modification of the DAG framework (as opposed to developing them from the structurally complex natural product ligands mentioned earlier).

Figure 19: PKC Isoforms in Cancer

CNS tumors	PKC α ↑, PKC γ ↑, PKC ϵ ↑, PKC ζ ↑, PKC δ ↓
Breast cancer	PKC α ↑, PKC β ↑, PKC δ ↓, PKC η ↓
Pituitary and thyroid tumors	PKC α ↑, PKC ϵ ↑
Leukemias	PKC β ↑
Skin cancer	PKC α ↑, PKC β ↓, PKC ϵ ↓, PKC ζ ↓
Colon tumors	PKC α ↓, PKC β ↓, PKC δ ↓, PKC ϵ ↓
Prostate cancers	PKC α ↑, PKC ϵ ↑, PKC ζ ↑, PKC β ↓

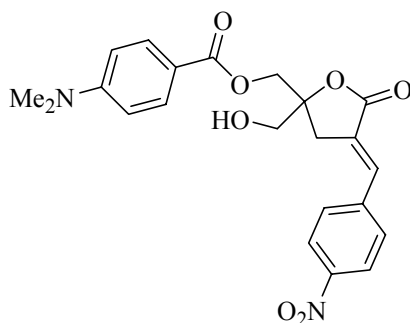
PKC activity can be modulated by:

- Changes in the residues at the DAG binding site in the C1 domain,
- Changes in C1 domain/other regions which interact with membranes, and
- Changes in membrane composition in various cells.

In previous work, the Marquez group has shown that flexible DAGs may be constrained via lactonization to give DAG lactones with potencies approaching those of natural products. Further, the identity of the side chain in DAG lactones determines biological function. In an example provided, DAG lactone HK 654 activated PKC α and induced apoptosis in LNCaP cells (human prostate cancer line).

More recently, the Marquez group has developed a combinatorial method for the synthesis of DAG lactones toward the development of PKC isozyme-specific ligands. Compounds prepared by this method were evaluated in a binding affinity assay using PKC α (and other PKC isoforms) for their ability to displace a tritium-labeled ligand PDBu from the enzyme. The DAG lactone **130C037** obtained by this method showed

Figure 20: 130C037



atypical binding curves for various PKC isoforms, with apparent K_i values ranging from 30 nM (PKC ϵ) to 350 nM (PKC α) for the five isoforms evaluated (Figure 20). In addition, **130C037** also bound RasGRP1 and RasGRP3 ($K_i = 3.5$ and 3.8 nM respectively), which represents an 8-fold and 90-fold selectivity versus PKC ϵ and PKC α (RASGrp family members also contain C1 domains activated by DAG). In LNCaP cells, **130C037** caused rapid translocation of RasGRP3, but only limited translocation of PKC ϵ , and no translocation of PKC α . It also induced ERK phosphorylation cells expressing RasGRP3, but not in control cells. The same compound was also able to differentiate between the C1b domains of PKC α and PKC δ ; it bound with good affinity only to δ C1b ($K_i = 1.78$ nM). The ability of **130C037** to discriminate between various enzymes with DAG-responsive C1 domains provides strong support for the feasibility of developing therapeutics targeted toward such enzymes.