Drugs of the Future 2010, 35(2): 143-153

CCC: 0377-8282/2010
DOI: 10.1358/dof.2010.35.2.1468927

MEETING REPORT

5TH DRUG DESIGN & LEAD DISCOVERY CONFERENCE 2009: LEAD FINDING STRATEGIES AND OPTIMIZATION CASE STUDIES

György M. Keserü

Gedeon Richter plc, POB 27, H-1475 Budapest, Hungary

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SUMMARY

This review reports the most important contributions of the 5th Drug Design & Lead Discovery conference, held in October 2009 in San Diego. The conference was focused on lead-finding strategies and compound optimization. Lectures covered state-of-the-art design strategies, application fragment-based approaches, the impact of ADME properties on lead finding and optimization, and a number of optimization case studies. In addition to the new technologies reported, both fragment-based and optimization case studies provided excellent examples for the lead discovery knowledge space that was discussed in detail during the conference.

INTRODUCTION

GTCbio organized the 5th Modern Drug Discovery & Development Summit in San Diego, USA, on October 14-16, 2009. In addition to the eight plenary lectures, the attendees could select from the seven tracks organized as parallel sections. These subconferences included the 4th Biological Therapeutics Research & Development conference, the 4th Translational Medicine conference, the 4th Partnering, Licensing & Outsourcing conference, the 5th Drug Design & Lead Discovery conference, the 4th Toxicity & Drug Safety conference, the Cancer Targets & Therapeutics conference and the 3rd Advances in Stem Cell Discovery & Development conference. Over 150 leading industrial and academic experts contributed to this 3-day summit. This overview is focused on presentations of the 5th Drug Design & Lead Discovery conference. Lectures of this conference can be grouped into four classes, including design strategies, fragment-based approaches, the impact of ADME properties and optimization case studies.

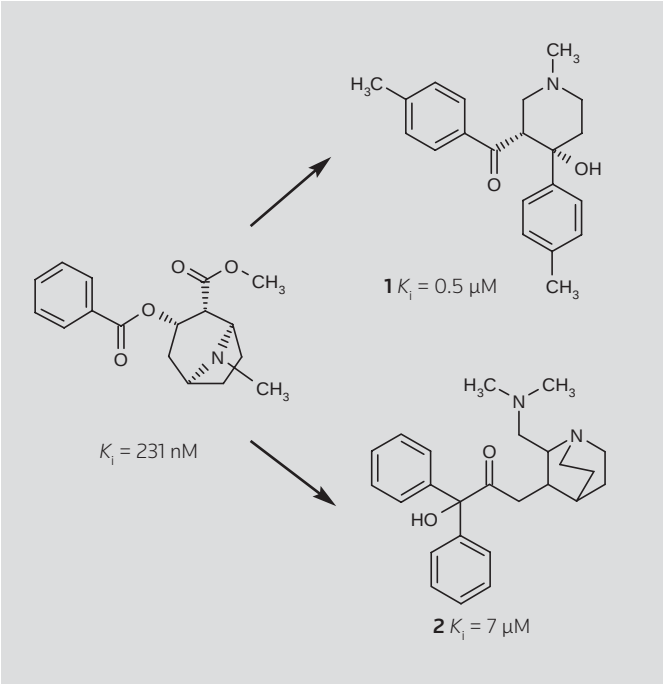
Correspondence: György M. Keserü, Gedeon Richter plc, POB 27, H-1475 Budapest, Hungary. E-mail: gy.keseru@richter.hu.

DESIGN STRATEGIES

Istvan J. Enyedy (Biogen Idec) gave a lecture on the application of pharmacophores in drug discovery. Pharmacophores are mainly used for hit identification and hit-to-lead (HTL) optimization, especially when no structural information on the target is available. In silico ADME predictions such as hERG and P-glycoprotein liabilities represent another important use for pharmacophore-based approaches. Specifically, they were found to be useful in virtual screening alone or in combination with structure-based approaches and scaffold hopping. The most important challenges of pharmacophores are the identification of bioactive conformations, the exploration and interpretation of multiple pharmacophores for a specific target and the rank ordering of hits obtained by pharmacophore searches.

The usefulness of the pharmacophore-based approach was demonstrated in a dopamine transporter (DAT) inhibitor program for cocaine addiction. Considering that the cocaine and dopamine binding sites are partially overlapped on the DAT, the objective of the optimization program was to identify compounds selective towards the cocaine binding site and with an improved pharmacokinetic (PK) profile relative to the competitor compounds. The project was started by screening the National Cancer Institute NCI-3D database including 206,876 compounds with a DAT pharmacophore developed from WIN-35065-2 and cocaine. The pharmacophore model consisting of a carbonyl oxygen, an ionizable nitrogen and an aromatic ring picked up two hits. One of these was a piperidinol (**1**) and the other was a quinuclidine (**2**), with DAT affinity of 0.5 and 7 μM, respectively.

The piperidinol scaffold was optimized, replacing the piperidinol moiety with a five-membered pyrrolidine ring, which provided a compound with DAT affinity of 1.4 μM that was further optimized to a compound with an affinity of 84 nM (**3**). The quinuclidine hit was



first fitted to cocaine and one phenyl group was removed and the ionizable nitrogen was replaced by an nBu chain to optimize their overlap, providing a compound with an affinity of 14 nM (**4**).

An alternative hit was identified by fitting mazindol to the previously developed DAT cocaine pharmacophore and minimizing nonoverlapping parts. The resulting compound (**5**) showed remarkable affinity towards DAT (24 nM) but was nonselective.

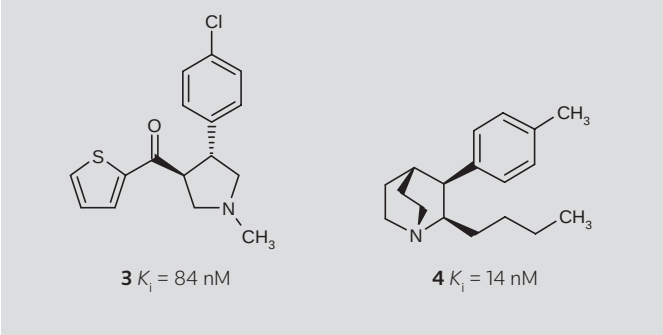
It was interesting to see that omitting one H-bond donor from the original pharmacophore resulted in compounds selective for the cocaine binding site of DAT. This observation underlies the finding of this group that successful scaffold hopping typically requires multiple pharmacophores.

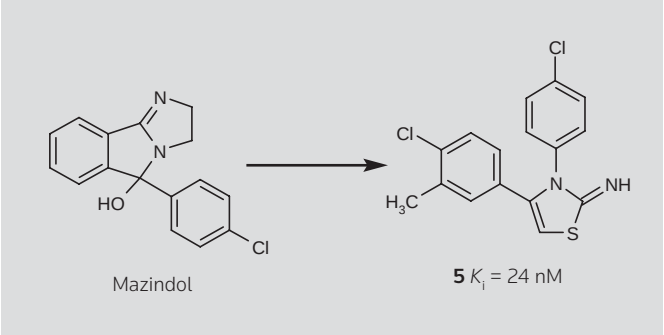
Pharmacophore-based virtual screening was reviewed by Hongwu Wang (Schering-Plough Research Institute). Pharmacophore features could come from one or more active compounds, as well as from x-ray structures of co-crystallized ligands. Basic pharmacophore features, such as H-bond donors, acceptors and hydrophobic contacts, can be extended by shape constraints, excluded volumes (from protein structures), topological models with defining functional groups, partial match of essential features and three-dimensional quantitative structure–activity relationships (3D-QSAR). These extended pharmacophores are typically used in virtual screening applications. Pharmacophore-based virtual screening includes model development, compilation of the 3D compound database to be screened, identification of primary hits by pharmacophore screening, clustering primary hits and testing cluster representatives.

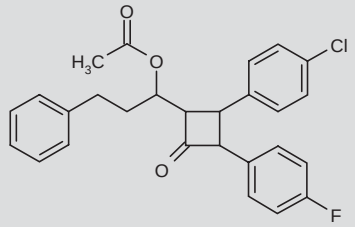
Pharmacophore models are derived from actives binding to the same pocket with the same mechanism but having diverse chemistry. These models should meet the following requirements: the model should pick up most of the actives in the training set; structural alignment used for the model should have some chemical sense; the number of pharmacophore features should be larger than four; and specific features are preferred over generic (a maximum of two hydrophobic features is allowed).

The 3D database of the screening library should be derived by extensive conformational sampling. These authors found that MacroModel (Schrödinger, Inc.) provided much better sampling than built-in approaches. Ionization and tautomerization of the screening compounds should also be considered. The screening database is compiled using multiple criteria, including availability, lack of reactive and undesirable moieties, rule-of-five compliance and occasionally hERG inhibition, cytochrome P450 inhibition, intestinal permeability, brain penetration and plasma protein binding criteria.

The effectiveness of this approach has been demonstrated in the virtual screening for a cannabinoid CB_1 receptor antagonist. The training set included eight CB_1 receptor antagonists/inverse agonists from the literature that were used to generate the pharmacophore hypothesis by Catalyst Hiphop. This five-feature pharmacophore was used to screen the Schering-Plough corporate database converted by MacroModel conformational search. The 23,000 primary hits were further filtered, selecting compounds with a molecular weight below 550, being available in 2 mg and fulfilling a modified rule of five. The resulting 7,200 compounds were filtered by a Bayesian classifier developed on 78 CB_1 receptor antagonists that gave 2,100 top-ranked compounds, which were clustered, giving 450 com-







6 K_i = 52.8 nM

pounds for biological testing. Finally, five compounds showed > 50% inhibition at 1 μM and one (**6**) showed a value of 52.8 nM for antagonism of the CB_1 receptor.

Deborah C. Reuter (Roche Palo Alto) discussed the impact of hydrogen bond acceptor strength on drug discovery. Hydrogen bond basicity (HBbasicity), either measured or calculated, has relevance for observed hydrogen bonding and SAR. Her team therefore developed a computational model for estimation of the acceptor strength. Their model was built from experimental values and molecular electrostatic potential, since there is a correlation between electrostatic potential and HBbasicity. Compounds were first converted to 3D by Corina and were then optimized by density functional theory (DFT) calculations using a 6-31G** basis set. Finally, electrostatic potential was calculated by the Jaguar ESP program. Two separate models were developed for oxygen- and nitrogen-containing compounds correlating the minimum electrostatic potential with the measured HBbasicity. The models were able to reproduce the trends observed in experimental data. These trends associated with hydrogen bond basicity and functional groups were analyzed. It was found that both cyclization and the introduction of electron-donating substituents increased HBbasicity. These models are useful for selecting HBbasicity "bioisosteres" that can be applied to lead optimization. On the other hand, a case study on MAP kinase p38 revealed that if HBbasicity correlates with affinity then it could be used for scaffold prioritization.

Holly Heaslet Soutter (Pfizer) discussed an antibacterial research program, the aim of which was to identify a potent inhibitor of dihydrofolate reductase (DHFR) from *Staphylococcus aureus*. The inhibitor needed to be potent against both the wild-type chromosomal form of DHFR and the drug-resistant, plasmid-encoded form of DHFR called S1. While structural information was useful in the optimization of ligand–protein interactions, there were no major differences in the binding mode between the wild-type and S1 forms of the enzyme that could explain the large differences in potency. A variety of biophysical techniques were used to analyze the molecular mechanism of S1 resistance. X-ray crystallographic analysis revealed that G43A and F98Y mutations were responsible for S1 DHFR trimethoprim resistance. Isothermal titration calorimetry (ITC) was used to analyze the binding thermodynamics of inhibitors towards the wild-type and the resistant forms of DHFR. ITC data measured for different isoforms revealed that the entropic factors were reversed in the mutant version relative to the wild-type protein. It was concluded that resistance to trimethoprim is due to a significant loss in binding enthalpy. Inhibitor-induced desolvation of the DHFR binding site was approximated by Watermap calculations. It was found that an entropically disfavored water is present in the wild-type binding site that is replaced in the resistant S1 version by the diaminopyrimidine core. This replacement might change the conformation of Phe92. Comparative nuclear magnetic resonance (NMR) studies on isotopically labeled DHFR and DHFR S1 revealed that it is the lid in S1 that moves significantly upon binding. Finally, it was demonstrated that DHFR S1 has faster on and off rates compared to the wild-type protein. This effect was confirmed for a number of inhibitors, and interestingly, off rates correlated with MIC data.

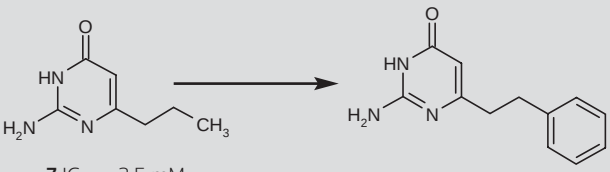
Combined investigations using x-ray crystallography, ITC, computational chemistry, protein NMR and binding kinetics measurements provided critical information for the optimization of S1 binding.

FRAGMENT-BASED APPROACHES

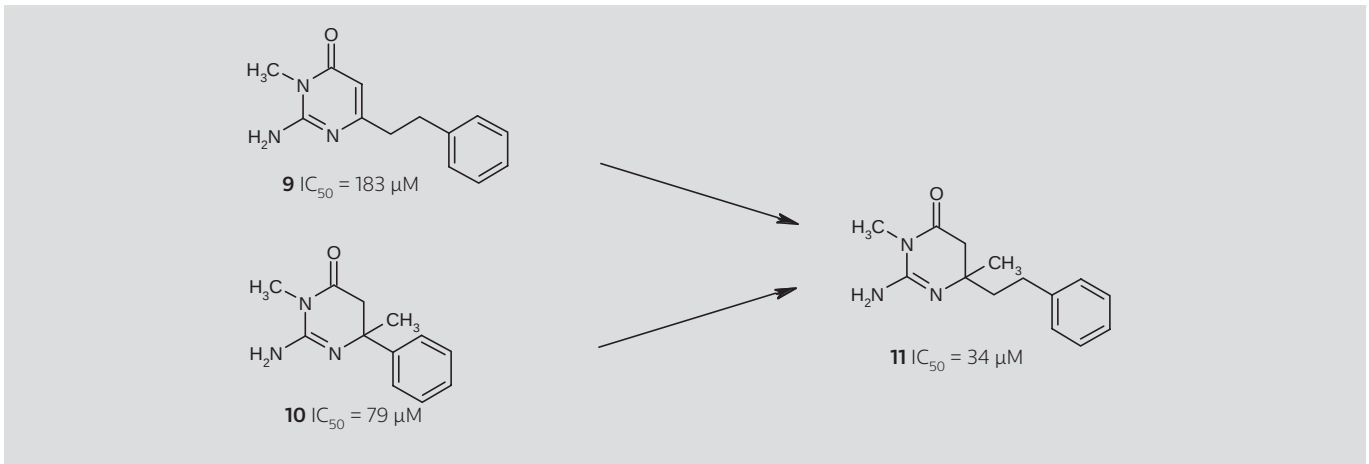
Jeffrey S. Albert (AstraZeneca Pharmaceuticals) reported the discovery of high-affinity β-secretase inhibitors using fragment-based lead generation. Fragment screening was initiated after the failure of several alternative lead generation strategies that included two high-throughput screening (HTS) campaigns using 10- and 30-μM screening concentrations, virtual screening, screening-focused libraries specifically designed against aspartic proteases and de novo design based on x-ray crystallographic analysis. All these efforts provided compounds with low activity and flat SAR and suboptimal ADME properties. As a parallel effort, Astex screened fragment libraries in collaboration with AstraZeneca. Surface plasmon resonance measurements validated a promising 2.5-mM hit (**7**) that was selected as a starting point for hit-to-lead optimization.

First, a similarity search identified 20 compounds having a similar core to that of the hit identified. Screening this set, the team was able to identify a compound (**8**) with an affinity of 570 μM and some basic SAR. This compound was co-crystallized with BACE1 and the binding mode suggested extending the compound towards the S2' pocket, which yielded an isocytosine compound with an affinity of 183 μM (**9**). The subsequent similarity search using this compound as query against the compound deck identified dihydroisocytosines (**10**) with remarkable affinity. Combining structural features of isocytosines and dihydroisocytosines, the team identified a compound with a potency of 34 μM (**11**).

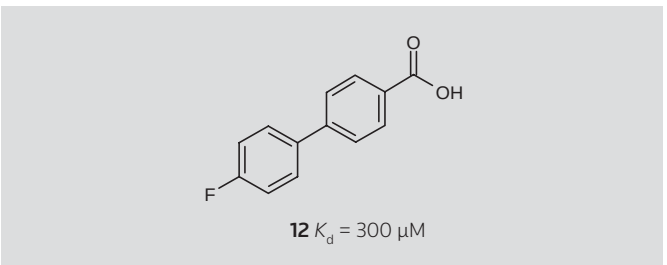
Further optimization of this series towards the S3 pocket of BACE1 led to an 80-nM lead with high ligand efficacy of 0.37 (Fig. 1).



7 IC_{50} = 2.5 mM **8** IC_{50} = 570 μM



Andrew M. Petros (Abbott Laboratories) reported the fragment-based discovery of Bcl-xL inhibitors. The program utilized NMR-based screening after the failure of the HTS campaign performed against the corporate compound deck. The lead discovery strategy followed the classical SAR-by-NMR approach that involved a first screening campaign for a binder, next focusing on identifying a second binder that binds in the presence of the first binder. Finally, these two binders could be linked, providing the lead. To achieve this goal, 10,000 compounds with a molecular weight of < 250 Da were screened in 1 mM, monitoring their binding by analyzing N15 heteronuclear single-quantum coherence (HSQC) spectra. This methodology enabled the identification of biaryl acids (**12**) with an extremely low false-positive rate.



The binding mode of this compound was explored by hot-spot analysis utilizing Ala scanning. This study revealed two major interactions, one with D38 (acidic moiety) and one with L78 (Ph moiety). Secondary screening was performed in the excess of a biaryl acid to target interactions to Ile85 located at the BAK peptide site of the target. Screening 3,500 compounds having a molecular weight of < 150 at 5 mM resulted in phenolic hits (**13** and **14**). The final step of

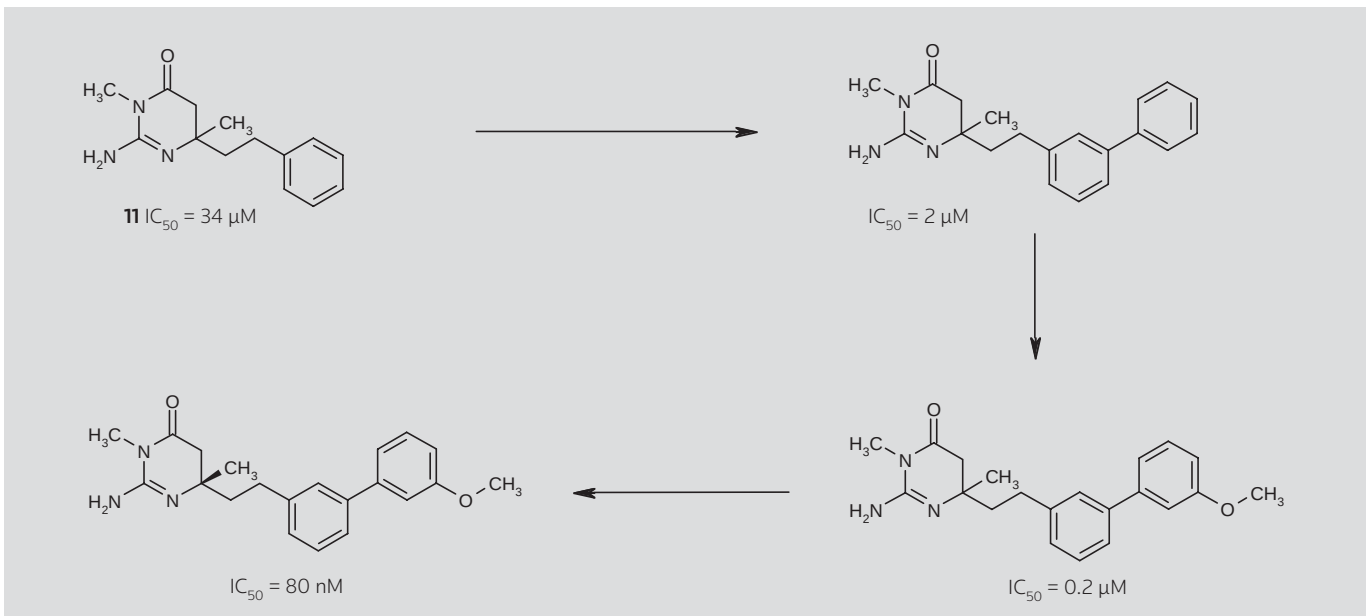
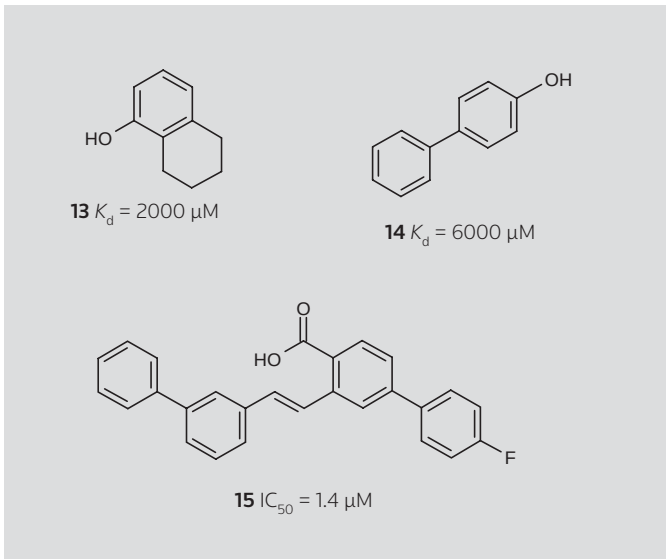


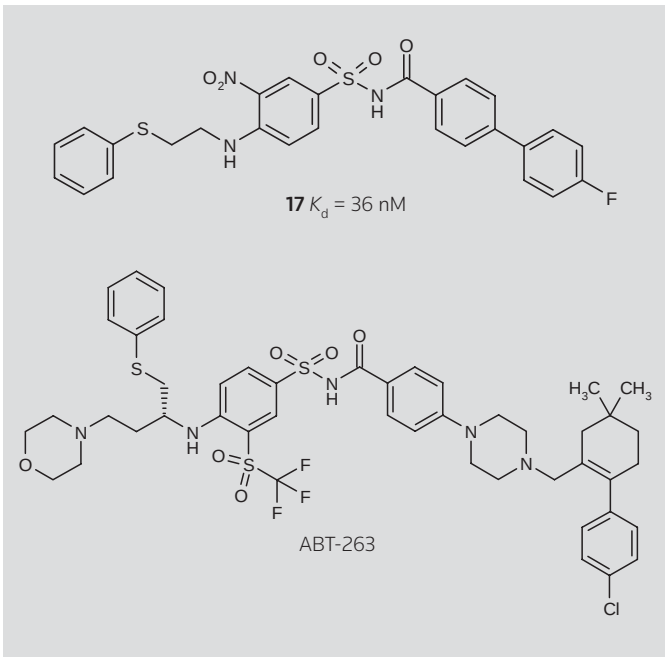
Figure 1. Optimization of isocytosines/dihydroisocytosines.



13 K_d = 2000 μM

14 K_d = 6000 μM

15 IC_{50} = 1.4 μM



17 K_d = 36 nM

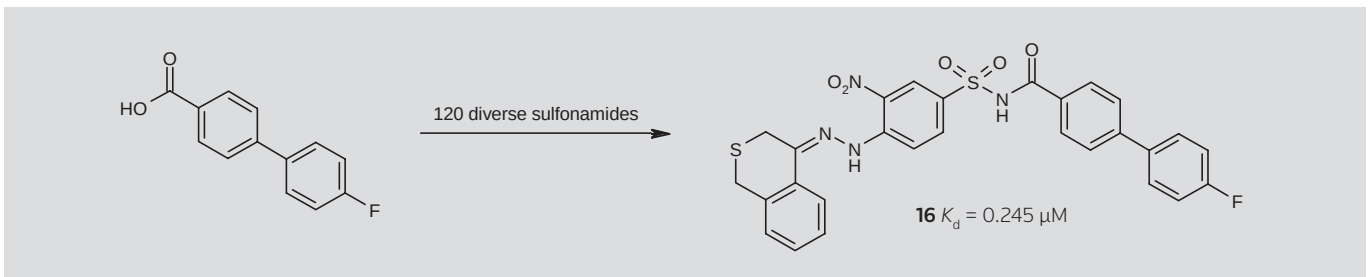
lead generation was to choose a suitable linker connecting the biaryl acid and phenol fragments (**15**).

The non-drug-like stilbene linker was then replaced by an acid sulfonamide linker and a diversity approach was applied for the identification of alternative second-site binders. These efforts resulted in a compound with submicromolar affinity (**16**).

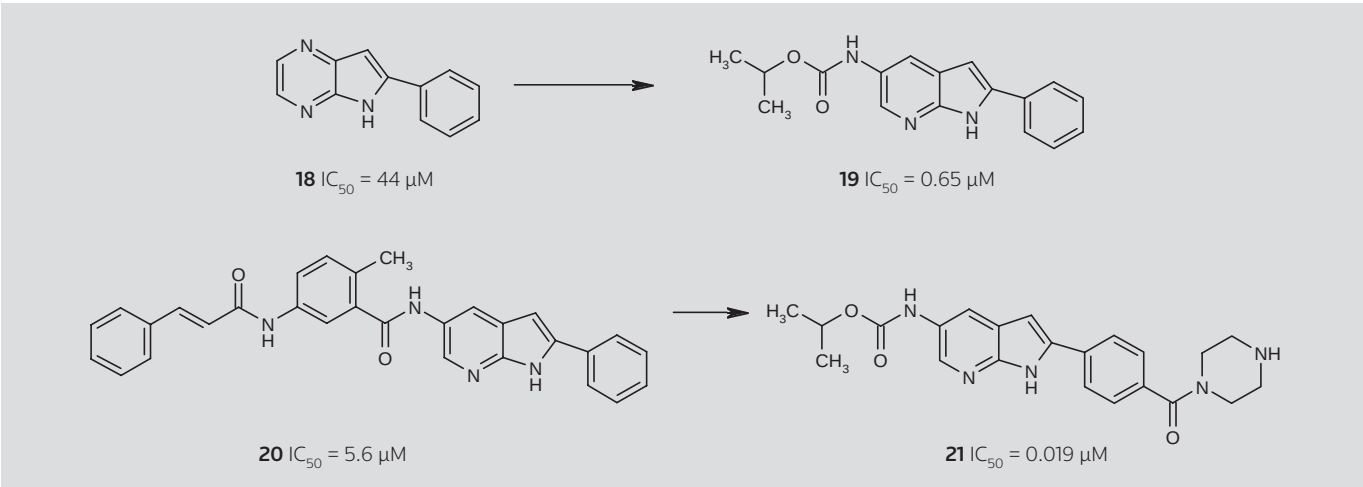
Further optimization at the second binding site yielded a potent lead with an affinity of 36 nM for Bcl-xL (**17**). Finally, this lead was converted to **ABT-263**, a potent, orally bioavailable inhibitor of both Bcl-xL and Bcl-2 which is currently in the clinic.

The most important issues for success in fragment-based lead discovery were summarized by Suo-Bao Rong (Pfizer Global R&D). The speaker identified the selection of fragments critical to identify fragment hits for the purpose of effectively exploring the target's binding pocket. Next, he concluded that it is crucial to characterize the binding pocket by fragment-binding experiments or computational fragment–protein simulations. The importance of the growing and linking strategy in a well-defined pocket and the preference for a fragment-linking solvent-exposed pocket were emphasized. Finally, it was emphasized that the success of fragment-based drug design is heavily impacted by the appropriate and integrated use of both experimental and computational fragment-based methods. These issues were demonstrated using an undisclosed protein–protein interaction target that was tackled by NMR-based fragment screening. Screening efforts yielded seven micromolar hits that were docked and prioritized on the basis of the binding modes predicted. Two of them showed a reasonable binding mode, while the other five were dropped, as the team concluded that these were unsuitable for structure-based design.

Sandor Vajda (Boston University) showed that computational fragment mapping, an in silico technology based on fragment docking, is useful for finding druggable sites in protein–protein interfaces (PPIs). Identification of small-molecule PPI inhibitors is challenging, since PPIs have several small pockets typically similar to those located in other regions of the protein, and some of the pockets may expand upon ligand binding. Computational fragment mapping algorithm (FTMAP) places small molecules or functional groups (molecular probes) on the surface of proteins in order to identify the most favorable binding positions. Application of mapping to a number of ligand-free PPI targets has shown that the method finds the site that can bind small-molecule inhibitors. The mapping algorithm involves soaking the PPI with rigid body docking of molecular probes, refinement of the docking poses, clustering poses and scor-



16 K_d = 0.245 μM



ing the clusters by molecular mechanics Poisson–Boltzmann surface area (MM-PBSA) function, and finally the identification of consensus sites using the centers of clusters. Side chains around the identified sites are then adjusted and are subjected to remapping that identifies the most important site and provides information on its druggability. The methodology was tested for a number of PPI targets, including IL-2, Bcl-xL, Mdm2, HPV-11 E2, zipA, TNF-α and NEMO. It should be noted that most of these applications are to relatively rigid proteins. It was concluded that FTMAP is capable of identifying the binding sites of organic solvents on proteins, in good agreement with x-ray and NMR data. They found that the consensus sites with large numbers of overlapping probe clusters indicate "hot spots" that can be considered as the most important regions of the binding site. On the other hand, sites with few probe clusters (typically less than 15) are not druggable. The methodology is available on the mapping server at http://ftmap.bu.edu.

Andreas Kuglstatter (Roche Palo Alto) demonstrated the impact of fragments on the discovery of kinase inhibitors using several case studies. Roche fragment screening technology incorporates surface plasmon resonance detection and x-ray crystallography. A typical workflow screens 5,000 compounds by surface plasmon resonance (SPR) that is followed by 7-point SPR measurements for actives, then prioritized hits are subjected to x-ray analysis. The fragment hits provided by this technology were used for rapid hit expansion, scaffold hopping, focused-library design, exploration of the protein flexibility and kinase selectivity design.

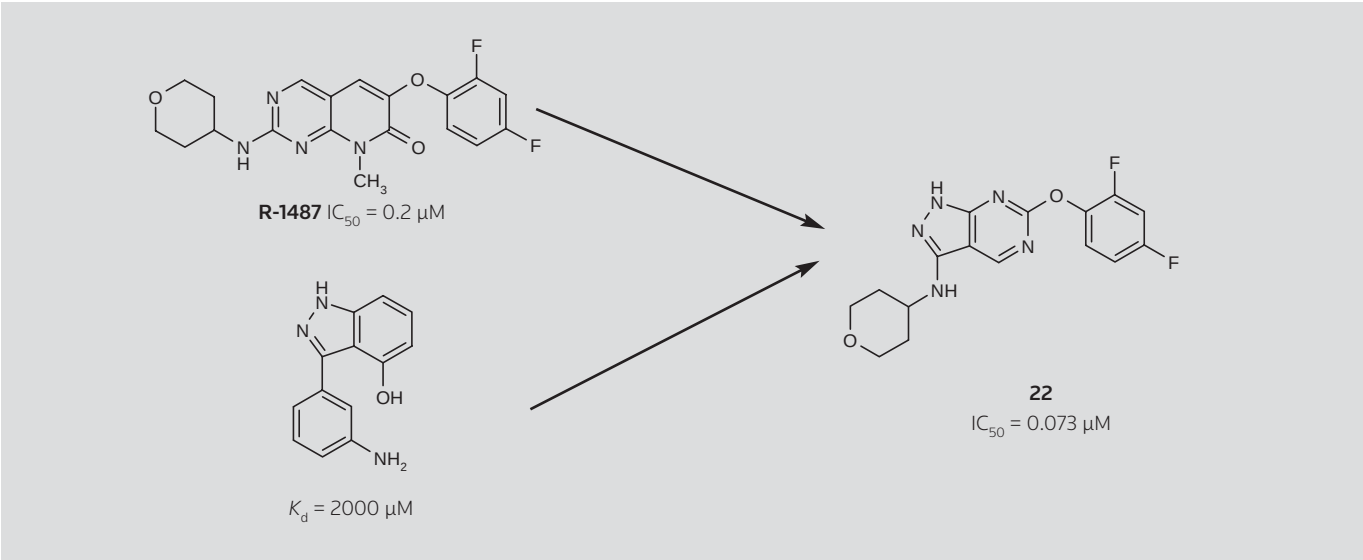
A hit expansion case study was presented on Bruton tyrosine kinase (BTK). Fragment screening identified 18 fragments with a K_d of 5-340 μM. These fragments were co-crystallized with the target and were subjected to x-ray analysis. It was concluded that a large variety of fragments all bound to the hinge region. A 130-μM compound (**18**; IC_{50} = 44 μM) was identified as a promising hit and 20 structural analogues from the corporate library were also investigated. One of these is a compound (**19**) that was extended towards the back pocket and demonstrated an IC_{50} of 0.65 μM. Another one (**20**) explored the DFG region, giving an IC_{50} of 5.6 μM, which represents potential for selectivity. Further optimization towards the front region led to a 19-nM lead (**21**) based within 3 months.

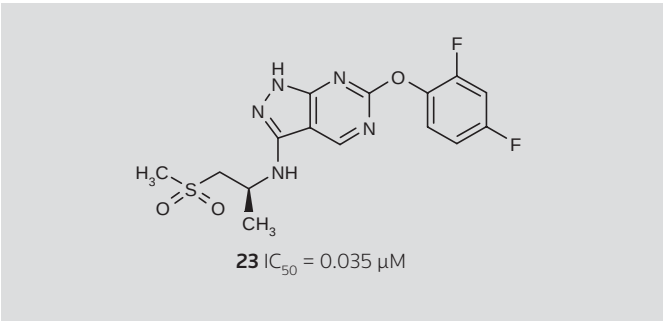
The application of fragment-based drug design for scaffold hopping was demonstrated on MAP kinase p38α. The team previously identified **R-1487** as a potent and selective inhibitor that is currently in phase II clinical trials and needs a backup. Fragment screening identified a 2-μM core that was inserted into R-1487, leading to a 73-nM compound (**22**) with a new scaffold.

Although the resulting compound had remarkable potency, it showed suboptimal properties, including low solubility and high lipophilicity. Increasing the solubility of compounds with high melting points was investigated by x-ray crystallography, which revealed pi–pi stacking interactions. It was hypothesized that low solubility is due to the high melting point (according to Yalkowski's generalized solubility equation), and therefore breaking pi–pi stacking might be a solution for improving solubility. A highly active compound with good solubility, improved logP and without pi–pi interactions was identified. The more potent enantiomer was selected as a clinical candidate (**23**).

Fragment hits were found to be useful for the design of focused libraries, as demonstrated on IRAK-4 and SYK kinases. Fragment screening identified 10 novel hinge-binding chemotypes and their binding modes were explored by x-ray crystallography. These fragments were used to design new, proprietary focused libraries. Design principles included: 1) template modification would be minimal 3D conservative; 2) R-groups should be limited to two to three diverse substituents; 3) novelty should be demonstrated as no hits in SciFinder; 4) properties should exceed the rule of five; 5) the exit vectors should indicate opportunities for multiple kinase conformations; 6) they should have reasonable synthesis; and 7) the focused library should contain 50-150 compounds plus fragments. A total of 1,978 compounds were synthesized and only 1 library had no hit when screened on 25 kinases internally (89% screened, 18% hit rate) and 402 kinases at a contract research organization (12% screened, 70% hit rate). These libraries could provide "off-the-shelf" hits for immediate follow up in subsequent HTL studies.

Exploration of protein flexibility was exemplified by JNK3 having very limited protein flexibility, as seen from 18 x-ray structures. Fragment screening, however, was able to identify a fragment that bound to the hinge region and stabilized a novel protein conformation.





Finally, the design of kinase selectivity was demonstrated on BTK. BTK was complexed with a broad range of chemotypes and the team identified three different protein conformations. Fragment screening identified a fragment that stabilizes a new protein conformation characterized by the activation loop forming a short α helix and collapses to the ATP binding site. This new protein conformation identified a ligand-binding subpocket that is specific for BTK and was used to design selective BTK inhibitors.

THE IMPACT OF ADME PROPERTIES

The presentation of György M. Keserü (Gedeon Richter plc) focused on how lead generation strategies impact the quality of leads. The speaker set up an analogy between human obesity and "lead obesity", defined as a condition characterized by increased molecular weight (analogous to increased body weight) and high lipophilicity (analogous to increased body fat) that may affect lead optimization and further development adversely. Analyzing 335 HTS-based and 184 alternative (natural product, virtual screening and fragment-based) HTL optimizations, it was demonstrated that lead obesity is independent of the lead discovery technology applied and the lead discovery library screened. The most striking finding was that even small and polar fragments were optimized to obese leads. Investigating the source of lead obesity, the speaker pointed out that lead optimizations driven by mostly entropic factors contribute significantly to lead obesity. Using a recent case study on small-molecule renin inhibitors developed by Pfizer, it was demonstrated that, contrary to entropic optimization resulting in obese leads, enthalpic optimization of the same HTS hit yielded a good-quality lead with an acceptable molecular weight and lipophilicity. The speaker concluded that structure-based design combined with monitoring binding thermodynamics could support enthalpic HTL optimizations. In addition, the use of proprietary reagent pools, carefully selected building blocks and specific chemistry knowledge might facilitate avoiding lead obesity.

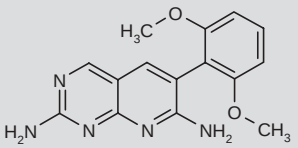
Mukesh K. Mahajan (GlaxoSmithKline) presented a new methodology for the detection and characterization of reactive metabolites. The speaker reported a dual-negative precursor ion scan approach which incorporates simultaneous scanning for the two commonly observed ion fragments from glutathione conjugates, m/z 272 and 254 (the dehydrated form of m/z 272). In the first case study discussed, the new methodology was compared to more traditional methods investigating diclofenac, carbamazepine, 3-methylindole and two proprietary GSK compounds. This case study demonstrated that the new approach has improved selectivity and sensitivity over existing methodologies and is particularly more efficient than single-precursor ion scans. The dual-negative precursor ion scan approach was useful for the reduction in false positives and total data analysis time. In the second case study, 28 substituted benzothiophene-containing compounds were screened for the formation of reactive electrophilic intermediates utilizing accurate-mass time-of-flight mass spectrometry. This study revealed that an electrophilic arene oxide intermediate is formed by the initial cytochrome P450-catalyzed epoxidation of the benzothiophene moiety, followed by nucleophilic attack of water or glutathione. The authors found that 18 of the 28 compounds (64%) formed byproducts of arene oxide intermediates and 12 of the 18 compounds indicated the arene oxide formation on the benzothiophene moiety, further implicating the involvement of arene oxide in potential bioactivation. The case study highlighted

the importance of evaluating metabolic pathways of new chemical entities (NCEs) as early as possible, particularly if benzothiophene or other known potentially reactive moieties are present. In these cases, the dual-negative precursor ion scan approach would provide benefits over existing methodologies.

Mette Guldbrandt (Novo Nordisk A/S) discussed the ADME properties specific for peptide drugs. Although currently the most common way of administering therapeutic peptides and proteins is by injection, there are significant efforts to use these drugs orally. To achieve this goal, however, several challenges must be overcome, including physical (permeation, transepithelial transport), chemical (acid hydrolysis, enzymatic degradation) and metabolic barriers (metabolic stability, clearance), as well as transit time and food effects. The ADME assessment of peptides with a molecular weight ranging from 1,000 to 6,000 might be significantly different from that used for small-molecule NCEs. Investigating physical barriers should include testing permeability, transport mechanisms (e.g., specific transport systems) and also absorption enhancers. Chemical barriers are typically involved in the degradation process. Therefore, the stability of the peptide/protein and the identification of degradation products are equally important. The metabolic characterization of peptides consists of testing metabolic stability and the mechanism of their clearance. The most important features for designing a reliable protocol include choosing relevant testing systems (tissue, in vitro model, in vivo setup), which requires extensive knowledge on the peptide/protein studied and also on the limitations of the assays. Bioanalytical support is essential for all in vitro and in vivo ADME studies. In the case of peptides, immunoassays are preferred over liquid chromatography/mass spectrometry (LC/MS) methodologies.

OPTIMIZATION CASE STUDIES

J. Richard Miller (Pfizer) showed three routes to novel antibacterial biotin carboxylase inhibitors. As part of its antibacterial drug discovery efforts, Pfizer screened a library of 1.6 million compounds to identify inhibitors of bacterial growth. Hits from this screen included



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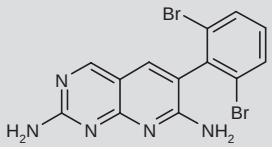

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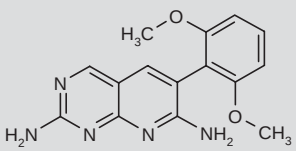
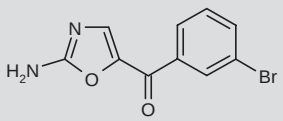
a class of pyridopyrimidines (**24** and **25**) with potent in vitro and in vivo activity against Gram-negative bacteria. This approach represents the classic HTS-based lead discovery.

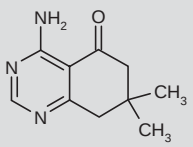
Further genetic and biochemical analysis links the antibacterial activity to inhibition of the biotin carboxylase component of acyl-CoA carboxylase. Identification of the target opened two new routes, including de novo fragment and virtual screening. Fragment screening was realized, as a high-concentration biochemical assay and saturation transfer difference (STD)-NMR were used to deconvolute individual hits. A 5,200-membered NMR fragment library was first tested in pools of 10 compounds by bioassay. Active pools further investigated by NMR screening identified 142 hits by NMR deconvolution of hit pools. Six of these hits (**24**, **26-30**) showed an IC_{50} of < 95 μM in the subsequent bioassay. The virtual screening approach used the high-resolution co-crystal structure of biotin carboxylase and a potent pyridopyrimidine inhibitor to drive a 3D shape and electrostatic similarity search of a 2.2-million compound library. IC_{50} values were determined for 525 hits and analogues, yielding a 10% hit



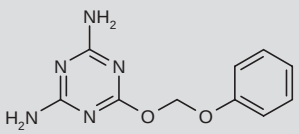
HTS hit **24**
IC_{50} < 0.01 μM



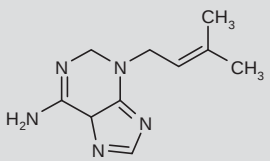
Virtual screening and nMR hit **26**
IC_{50} = 21.5 μM



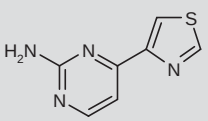
NMR hit **27**
IC_{50} = 60.8 μM



Virtual screening hit **28**
IC_{50} = 25.4 μM



Virtual screening hit **29**
IC_{50} = 1500 μM



Virtual screening hit **30**
IC_{50} = 12.0 μM

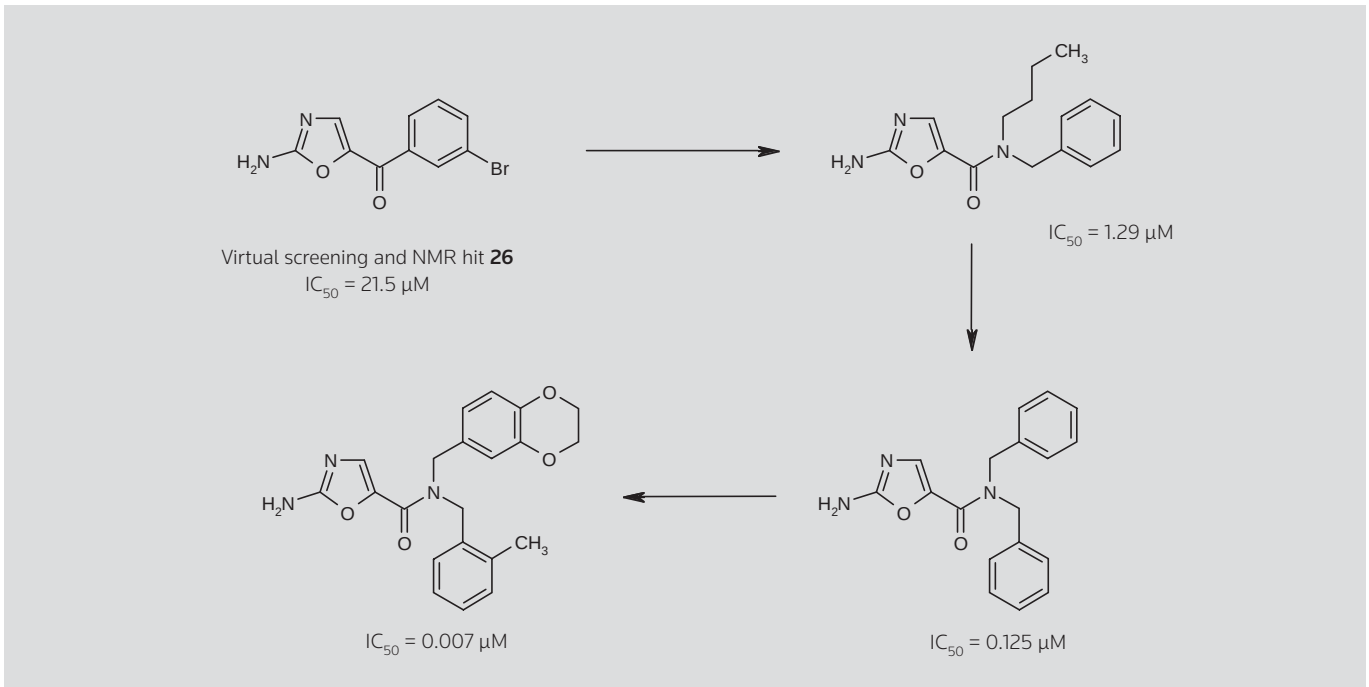


Figure 2. Lead series with mechanism-based antibacterial activity.

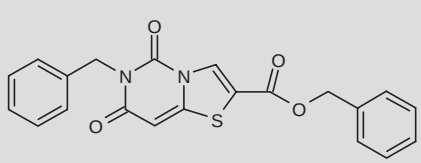
rate for the virtual screening approach that was found to 200-fold higher than that of the HTS approach. Combining experimental and virtual approaches led to the identification of several ligand-efficient fragments that inhibited biotin carboxylase.

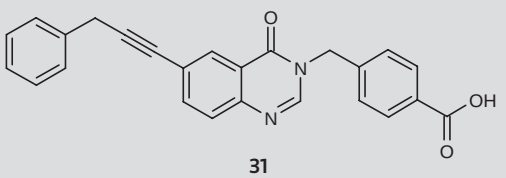
Through iterative rounds of structure-based drug design, one fragment/virtual screening hit ultimately led to a novel lead series with mechanism-based antibacterial activity (Fig. 2).

Specific matrix metalloproteinase MMP-13 inhibitors for the treatment of osteoarthritis were presented by Jie Jack Li (Bristol-Myers Squibb), as realized at Pfizer's Ann Arbor site. An HTS campaign against MMP-13 identified **PD-503**, which was found to be specific towards MMP-13 and did not chelate the central Zn atom, as observed for nonspecific inhibitors.

The thiazolopyrimidinone core of the original screening hit was replaced by a quinazolinone moiety and optimized at the S1′ site, yielding a series of highly potent and selective compounds exemplified by compound **31**. This compound was active in vivo and caused no side effects in the rat musculoskeletal side effect model. The main issue with compound **31** was that a potential reactive metabolite could be formed from the alkyne moiety. The alkyne was therefore replaced by an amide, as suggested by structure–activity data obtained on nonspecific MMP inhibitors. Compound **32** showed high affinity and selectivity, reasonable ADME properties and acceptable PK. PF-212, an undisclosed representative from this chemotype, demonstrated efficacy in vivo and was investigated in toxicological studies in monkeys, where it showed kidney toxicity. The project was transferred to the St. Louis site and further optimization took place there.

Mark Smith (Roche Pharmaceuticals) presented results on the design, synthesis and antiviral potency of 4′-substituted nucleosides. The discovery of 4′-azidocytidine **R-1479** (IC_{50} = 0.171 µM in a genotype 1b replicon assay), a potent inhibitor of RNA synthesis by


PD-503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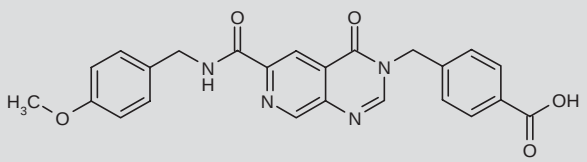
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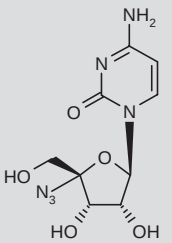
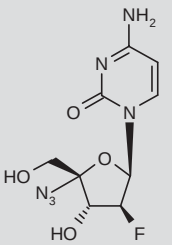


R-1479



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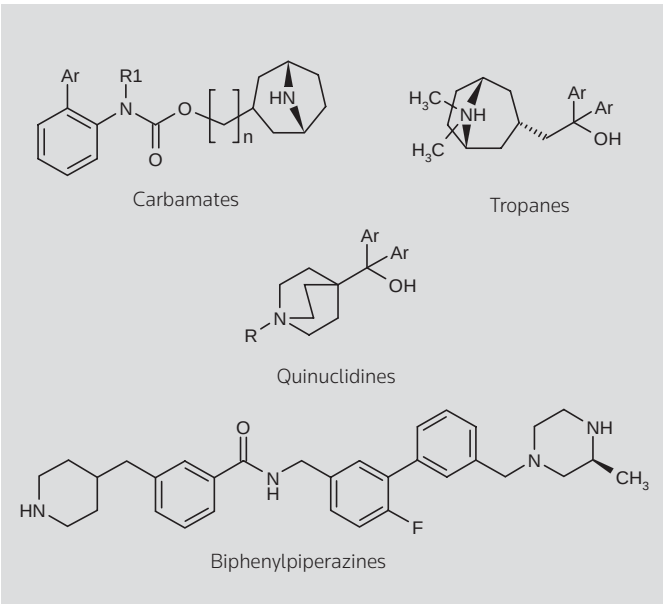


Figure 3. Carbamates, tropanes, quinuclidines and biphenylpiperazines as muscarinic M_3 antagonists.

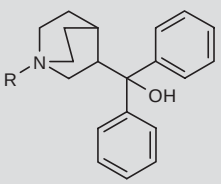
NS5B (EC_{50} = 1.28 μM), the RNA polymerase encoded by hepatitis C virus (HCV), has led to the synthesis and biological evaluation of several derivatives of 4′-azidocytidine, varying the substituents at the ribose 2′- and 3′-positions.

2′- And 3′-substituted compounds with F and/or OH substituents were designed and synthesized as the most promising compounds. The most potent compound in this series (**33**) showed an IC_{50} of 0.024 μM in the genotype 1b replicon system.

Dramane Laine (GlaxoSmithKline) presented the discovery of new long-acting muscarinic antagonists for the treatment of chronic obstructive pulmonary disease (COPD). In COPD airways hyperreactivity is mediated by a loss of cholinergic neural control of airways smooth muscle, leading to increased stimulation of M_3 muscarinic acetylcholine receptors (mAChRs). Thus, blockade of M_3 mAChRs is a useful therapy for COPD. The program at GSK was initiated with the goal of identifying compounds suitable for once-daily administration via inhalation for the treatment of COPD. The team used design principles balancing between potency (nM–pM), safety (limited systemic exposure, low F% and high CL) and kinetics (lung retention for dose-related 24-h duration of action). The screening cascade was therefore focused early on duration of action and efficacy (using an in vitro binding assay and mouse COPD model), and other profiling was done in parallel but not in the critical path. The lead-generation strategy included multiple technologies, including data mining of the GSK database (cross-screens and selectivity assays), rational design based on homology models (in-house and literature) and HTS. Combined efforts identified four chemical series: carbamates, tropanes, quinuclidines and biphenylpiperazines (Fig. 3).

This presentation focused on the optimization of the quinuclidine series. HTL optimization was started from a compound with micromolar affinity towards M_3 receptors. Optimization of the substituents of the quinuclidine core showed that a 1,4-substitution is beneficial. Substitution at the charged nitrogen atom also increased the M_3 affinity. These efforts led to the identification of an initial lead (**34**) with an IC_{50} of 87 nM. Since it was demonstrated that the formation of the quaternary salt increased the duration of action, further optimization was focused on quaternary nitrogen substituents. The most promising compound in this series was compound **35**. The in vivo acetylcholine-induced bronchoconstriction assay in mice revealed that compound **35** has a dose-dependent duration of action. This was also demonstrated in human tissue by measuring the recovery of carbachol-induced contractions in human bronchus. Furthermore, M_3 binding kinetics showed slow reversibility for the compound. The pharmacokinetics (low bioavailability and high clearance) were best suited for inhalation and supported the selection of this compound for clinical development.

Hui Li (Pfizer Global Research & Development) discussed the discovery of potent and selective PKCβ inhibitors for diabetic complications such as diabetic nephropathy, retinopathy and neuropathy. PKC inhibitors could provide an opportunity to control chronic hyperglycemia in diabetes, as was demonstrated for ruboxistaurin, a selective PKC inhibitor from Lilly that showed efficacy in phase II/III



34 R = $-CH_2CH_2Ph$; IC_{50} = 87 nM
35 R = $-(CH_2)_2OCH_2Ph$; IC_{50} < 10 nM

clinical trials for both diabetic retinopathy and nephropathy. On the other hand, the development of a PKC inhibitor would be challenging, since high selectivity and good safety are needed because treatment is chronic. The highly homologous structure of the members of the gene family makes the program even more complicated. HTS of Pfizer's corporate library picked up a pyrrolopyrazole hit having reasonable affinity for PKC (500 nM), but having a high molecular weight, high logP, high clearance, low permeability and low ligand efficacy. The ATP-competitive hit represents a unique hinge-binding moiety; its structure was nonplanar, with doable chemistry and good solubility. Similar compounds showed affinity towards Aurora kinase and CDK2. The optimization strategy involved the identification of the minimum structure required for potency. Modifications at the hinge-interacting part markedly decreased the potency. Head and tail regions, however, could be optimized, identifying compounds with single-digit nanomolar potency in both biochemical and cellular assays. ADME optimization of this series was performed, balancing among potency, permeability and in vitro metabolic stability. Basic physicochemical parameters were used to design compounds with an acceptable ADME profile. cLogP was set between 2 and 3.5, maintaining acceptable permeability and human liver microsome stability. Permeability is influenced by multiple factors. Analyzing polar surface areas and pK_a dissociation constants, the team concluded that good permeability requires a total polar surface area (TPSA) < 110 and a pK_a < 9. A p*K*a > 6 was found to be necessary for good potency, indicating an optimal pK_a range between 6 and 9 for this series of compounds. Rational use of intramolecular H-bonds helped govern the pK_a to the optimal range. In addition to the identification of the adequate physicochemical space, the influence of stereocenters on potency was also analyzed. In the absence of x-ray crystallography information, good general kinase selectivity and PKC isozyme selectivity were also achieved through introduction of key stereogenic centers. Pyrrolopyrazoles showed in vivo efficacy, as demonstrated in streptozotocin-treated rats by measuring PKC levels.

CONCLUSIONS

The 5th Design & Lead Discovery conference provided an excellent opportunity to identify the most recent developments in the field. Leading pharmaceutical companies presented a large number of case studies on both fragment-based lead generation and traditional HTL optimization. These experiences support the general efforts of the medicinal chemistry community, making lead generation more successful in terms of both quantity and quality.

DISCLOSURES

The author is an employee of Gedeon Richter plc.