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Research Concepts of Swiss Startup Companies

Mini-Symposium of the Division for Medicinal Chemistry (DMC) of the Swiss Chemical Society (SCS), at the Institute of Organic Chemistry, University of Basel, May 13, 2004

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Introduction

In recent years pharmaceutical research in Switzerland has diversified considerably due to the dynamic activities of several start-up companies. In the following overviews, representatives of seven Swiss companies outline their research activities. The sequence follows the order of speakers at the mini-symposium.

Thomas Rückle (Serono Pharmaceutical Research Institute, Geneva) presented a lecture on 'Medicinal Chemistry in the Fast Changing World of Biotechnology'.

In the late 90's, Serono's management made the strategic decision to add small molecule drug discovery to their research portfolio with the aim of mimicking and further extending the spectrum of action of the existing protein therapeutics with orally bioavailable next-generation products. As a hallmark of this new research paradigm, in 1998, Serono established a new, state-of-the-art Chemistry Department within the newly created research center in Geneva (Serono Pharmaceutical Research Institute, SPRI) with the necessary manpower, expertise, as well as the medicinal, analytical, and combinatorial chemistry equipment including extensive structure- and ligand-based design capabilities.

The mission of Serono Discovery Chemistry is to focus on the design synthesis and characterization of new molecules to be developed as medicines in close collaboration with Serono biologists and pharmacologists in the areas of reproductive health, metabolic endocrinology, cancer, neurology, and immunology.

In conjunction with a Chemistry Department previously established at Serono's Boston-based research site SRBI (Serono Reproductive Biology Institute), chemistry teams have a strong commitment to discover innovative therapeutic treatments and ultimately address unmet medical needs by taking advantage of the novel avenues offered by Serono biotechnology expertise. Serono has the ambition to 'explore the unexplored' in order to address the new challenges of genomics and proteomics in drug discovery. New concepts are constantly elaborated and discussed and new technologies are implemented in order to face the challenges of the post-genomic era. Such concepts, tools, and technologies are at the frontier of chemistry (synthetic combinatorial, medicinal, analytical and computational chemistry), cheminformatics, HTS technologies, structural biology, cell biology, and pharmacology.

Serono chemists have now been working for around five years on a variety of small molecule drug discovery projects. As the first small molecules emerging from these efforts have started entering human clinical trials, the presentation held at the Division for Medicinal Chemistry Symposium in Basel in May 2004 gave an account on three different exemplary approaches applied to some of the projects.

Firstly, an oxytocin–receptor (OT-R) antagonist program is illustrated as an example of established targets. GPCRs have proven to be remarkably successful targets for pharmacological intervention. Potent and selective small molecules with

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appropriate drug-like properties have been developed against a wide variety of GPCRs, acting as antagonists, partial agonists or full agonists. At an early stage within Serono, with its remarkable history in reproductive biology having culminated in several marketed protein therapeutics (*e.g.* Gonal-F, Luveris, Ovidrel), a number of GPCR targets involved in human reproduction were selected for drug discovery programs, one of which was the OT-R.

Oxytocin (OT), a nonapeptide, stimulates contractile activity in human myometrium, both *in vivo* and *in vitro*, and is widely used alone or in combination with prostaglandins for the induction of labor. Given its biological function, the OT-R has long been recognized as a prime pharmacological target for the treatment of preterm labor, a condition often leading to premature birth, which remains a major problem in obstetrics affecting about 10% of all births, making it the largest cause of perinatal morbidity and mortality [1]. Proof of concept comes from the peptide OT-R antagonist Atosiban, marketed in Europe under the trade name of Tractocile, which was shown to be efficacious in the treatment of imminent preterm birth. However, its peptidic nature requires constant infusion. We therefore set out to develop non-peptide, orally active OT-R antagonists with a better selectivity profile towards the vasopressin receptors.

High-throughput screening of a GPCR-directed combinatorial library led to the identification of an unusual proline-derived oxime ether compound, showing good affinity to the human OT-R (K_i = 260 nM). Hit-to-lead chemistry has optimized to a series of selective OT-R antagonists active *in vivo* (uterine contraction), out of which emerged several compounds with an overall improved biopharmaceutical profile [2]. Today, one compound has been advanced to human clinical trials, backed up by several follow-up compounds currently progressing in late-stage preclinical development.

The ***second case*** illustrates a kinase inhibitor program, as an example of how Serono uses computational tools to identify privileged chemotypes for certain target classes, how this can be extended to properties other than biological activity and how this can lead to the emergence of new chemical series by understanding the selectivity profiles.

Protein kinases and phosphatases are the key components of intracellular networks that use protein phosphorylation to transmit signals. In consequence, these proteins are important targets for new drug discovery as they play a critical role in a wide range of disease states such as inflammation, diabetes, cardiovascular and neurological disorders. Initially we were interested in MAP-kinases in particular c-Jun N-terminal Kinase (JNK), which has shown to play a critical role in a wide range of diseases including cell death (apoptosis)-related disorders (neurodegenerative diseases, brain, heart and renal ischemia, epilepsy) and inflammatory disorders (multiple sclerosis, rheumatoid arthritis, inflammatory bowel diseases). Screening of our compound collection for inhibitors of JNK3 identified several promising starting points that were subsequently optimized for potency, selectivity, and biopharmaceutical profile [3–6]. One compound emerging from these efforts entered human clinical trials earlier this year, and several back-up molecules are currently progressing in late-stage preclinical development. In the course of this program several non-selective JNK-inhibitors could be identified, which instead of being discarded were carefully analyzed using a proprietary, gradient-based substructure-identification process termed 'discrete sub-structural analysis'. This Serono-tool allows us to be 20- to 100-fold more effective than random screening. Several new proprietary chemical classes as inhibitors for different kinases came out of these efforts.

Finally, in a ***third example*** efforts in a kinase project with the aim to identify orally active isoform-selective PI3Kγ-inhibitors are highlighted. This represents a case where structure-based design and X-ray crystallography have shown that such techniques can support and boost medicinal chemistry programs in tackling the challenges of isoform selectivity.

Phosphoinositide-3-kinases (PI3Ks) are a family of lipid kinases that can be classified into three subfamilies according to their structure and substrate specificity [7]. Of those, the most extensively studied are the class I PI3Ks, and are further subdivided into class IA (PI3Kα, PI3Kβ, PI3Kδ) and class IB (PI3Kγ). The expression of PI3Kγ is mainly restricted to leukocytes. Inhibition of PI3K is thought to represent yet another way to interfere with the chemokine network. Today there is overwhelming experimental evidence that PI3Ks, in particular the γ- and δ-isoforms, play a critical role in the innate and adaptive immune response; a view that has been borne out by several studies involving genetically modified mice [8]. With all this in mind, a PI3K-inhibitor program was established at Serono. Primary screening of kinase focused sets led to the identification of a selective inhibitor that showed good activity against PI3Kγ (70 nM). Extensive use of crystallographic data plus molecular modeling have had great impact on the progress of hit to lead chemistry in improving potency on PI3Kγ by factor of 100, while constantly maintaining an acceptable oral biopharmaceutical profile. Endowed with excellent pharmacokinetic properties in mice and rats, compounds were further shown to be orally active in several murine models of inflammation.

In ***summary*** the different approaches described in the presentation illustrate the small molecule drug discovery strategy currently being applied at Serono. The way to discover drugs is changing very fast, mainly because new technologies have emerged that have changed the landscape, and time has come to move to the next generation of drugs. The initial spirit of being a start-up unit within an important biotech company could be conserved in a fully integrated drug discovery process of Serono.

Philipp Ermert (Polyphor, Allschwil) presented 'Substance Libraries for Lead Finding and Optimization'.

Polyphor was founded in 1996. Today, the company has 62 employees providing the research-based pharmaceutical and chemical industry with innovative, high-quality products in the field of lead finding and optimization. Polyphor offers a continuously growing general library of small molecules, currently comprising 25,000 pure compounds derived from over 500 scaffolds. Polyphor's proprietary protein epitope mimetic technology is aimed at finding hits and leads targeting large surface protein-protein interactions [9].

Polyphor also provides focused libraries based on scaffolds proposed by customers. For example, a collaboration between Polyphor and Roche (UK) was aimed at the synthesis and evaluation of a new class of influenza endonuclease inhibitors [10].

The influenza endonuclease is a key component of the viral transcription initiation mechanism which has no cellular counterpart and should therefore provide strong potential for discovering selective non-toxic drugs. By blocking the viral transcription, such compounds would complement existing therapies based on neuraminidase inhibitors which prevent the release of newly formed virus particles. The endonuclease domain of influenza RNA polymerase belongs to the group of phosphate processing enzymes containing two metal ions. 2,4-Diketobutanoic acids **I** and N-hydroxyimides **II** were identified as inhibiting the endonuclease activity. The compounds of both series are potential chelators of divalent cations. Based on molecular modeling studies [10], the Roche group proposed tetramic acids **III** (combining the structural features of **I** and **II**) as novel endonuclease inhibitors and selected 144 derivatives to be prepared.

The synthesis outlined in the Scheme is described in more detail elsewhere [10]. Key elements were the partial reduction-cyclization sequence converting the nitrophenyl acetic acid **2** into the (protected) hydroxy indolinone **3**, and the introduction of the tetramic acid moiety by a Knoevenagel type condensation. A suitable protective group strategy was required, allowing the purification of the fully protected targeted compounds and offering a simple deprotection procedure which facilitated the isolation and purification of the acidic, highly polar products **IIIa-c** in parallel fashion.

Within four months, 131 derivatives were obtained in an average quantity of 38 mg and an average HPLC purity of 92%. From the 131 member library, nine compounds showed an $IC_{50} \leq 10$ μM (which compares well with the potency of known inhibitors); six of these compounds were tested in cell culture and compound **7** showed the best antiviral activity. There was a clear preference for the low variability substituent R^1 to be phenyl rather than methyl or ethyl.

Laurenz Kellenberger (Basilea Pharmaceutica, Basel) gave an overview on 'Anti-Infective Research at Basilea'.

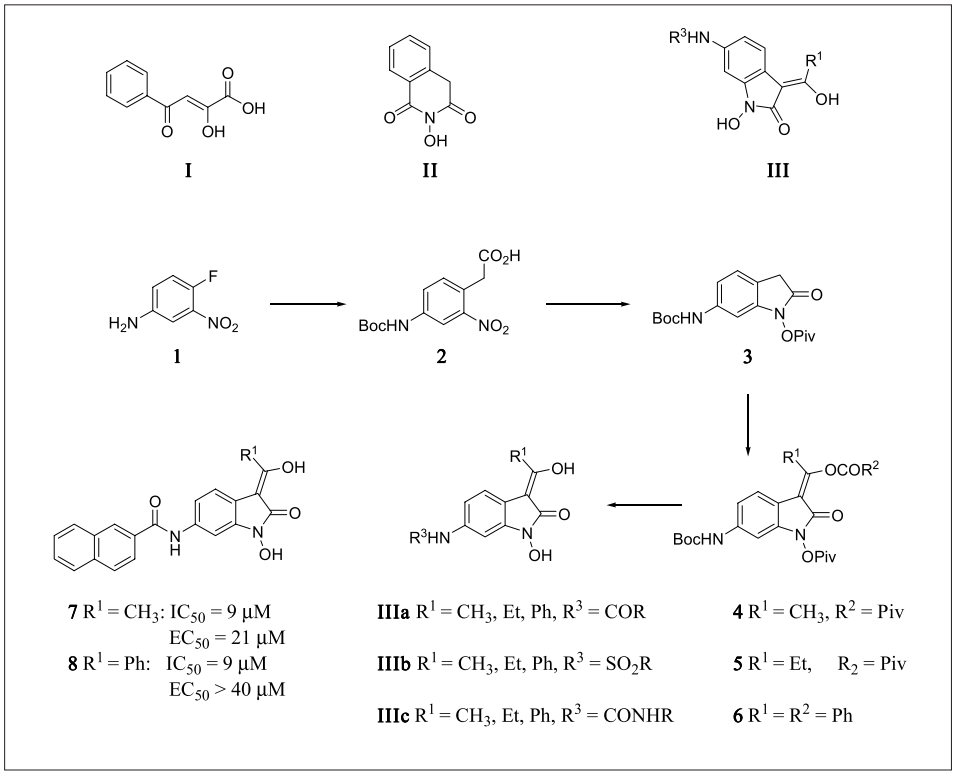
Basilea Pharmaceutica AG, with headquarters in Basel, is an independent biopharmaceutical company founded in October 2000 following a strategic decision by Roche to spin-off its R&D activities in the antibacterial, antifungal and dermatology therapeutic areas. At its foundation Basilea received significant assets to achieve its business objective of discovering and developing innovative medicines for the treatment of unmet medical needs. These assets included strong preclinical and clinical project portfolios and the antibacterial and antifungal intellectual property portfolios (IP) of Roche, as well as a substantial portion of its dermatology IP. Basilea commenced operations with 48 employees, including key scientists who invented many of the company's current research and development compounds. Key functionalities such as drug supply and drug development were added subsequently and today Basilea has fully integrated R&D operations. In its successful IPO in March 2004 the company raised CHF 206 million to support current and future R&D activities.

Basilea currently has three compounds in clinical development: an anti-bacterial, an anti-fungal and a dermatology compound. BAL5788 is the first of a new class of broad-spectrum cephalosporin antibiotics that has a unique combination of features resulting in potent bactericidal activity towards important pathogens, in particular methicillin-resistant *Staphylococcus aureus* (MRSA). BAL5788 is anticipated to enter phase III clinical development in the second half of 2004. In the field of dermatology, Basilea has completed phase II clinical development of its oral retinoid BAL4079, a compound that could become the first approved treatment for refractory chronic hand dermatitis. BAL8557 is a water-soluble azole that can be given oncedaily orally or as an injection for the treatment of serious fungal infections.

The expertise of the Basilea research team covers the whole drug discovery value chain including chemistry, biology, and pharmacology. In addition, a wholly owned subsidiary company in China provides Basilea with complementary chemistry and process research services to support research and development projects.

The anti-infective research strategy of Basilea is currently focused on the identification of new compounds able to overcome the increasing problem of drug-resistant bacterial infections, with the emphasis on difficult-to-treat pathogens that are already or are likely to become resistant to currently used antimicrobial agents. *S. aureus* is a prime example of such an organism: in the early 1940s, when penicillin G was introduced to the market, virtually all strains of *S. aureus* were susceptible to this β-lactam antibiotic, but by 1944, penicillinase-producing strains of *S. aureus* had started to accumulate in hospitals and today over 80% of clinical isolates are resistant to penicillin G [11]. Whereas drug-resistant bacteria such as MRSA used only to occur in hospitals, they are increasingly being observed in community settings.

Pathogenic microorganisms have responded to the widespread use of antibiotics by activating or developing a plethora of resistance mechanisms. The major mechanisms for resistance are inactivation of the antibiotic, alteration of the target site, prevention of access to the target, increasing efflux of the drug, and bypass mechanisms. Basilea's research efforts are directed at the identification of new agents able to overcome a microbe's resistance mechanism(s). Our main approaches are (i) modification of underexploited antimicrobial compound classes, (ii) discovery of novel classes of antibiotics directed at novel microbial targets and (iii) identification of novel molecules from successful antimicrobial compound classes able to overcome resistance. The cephalosporin BAL5788 exemplifies the latter approach. β-lactam antibiotics kill bacterial cells by inhibition of transpeptidases, which are enzymes involved in cell wall biosynthesis. Staphylococci have become resistant to β-lactams not only by producing β-lactamases (*i.e.* hydrolases), but also through the expression of an entirely new transpeptidase (PBP2') that can still make cross-bridges in the cell wall, but resists inhibition by the antibiotic. In BAL5788 the cephalosporin side chains have been modified and optimized in a



Scheme.

way that the compound exhibits increased stability towards β-lactamases and is again able to inhibit the new *S. aureus* transpeptidase PBP2'. The inhibition of transpeptidase is a three-step process proceeding via association, acylation of the enzyme and finally hydrolysis to free up the active site of the enzyme and release inactive β-lactam [12]. It could be shown that BAL5788 achieves potent inhibition of PBP2' through high affinity, a high rate of acylation, and a low rate of deacylation, thus reversing all of the factors that normally make this protein resistant to β-lactams [13].

Alex Räber (Prionics AG, Schlieren) spoke on 'BSE Diagostics: From Surveillance to Food Safety'.

The market leader for rapid prion testing – Prionics – was founded in 1997 as a spin-off from the University of Zurich. Prionics carries out research and development in prion diagnostics, preventive and therapeutic approaches to prion diseases and basic research in revealing the function of the prion protein. The combination of outstanding research, high-quality products and new marketing ideas is the recipe for success of this company, recipient of the Swiss Economic Award and designated as 'Company of the Year' in 2002. Prionics' main products are the rapid tests for diagnosis of transmissible spongiform encephalopathies (TSE) belonging to the Prionics®-Check product family providing tailor-made solutions for the specific needs of diagnostic laboratories. The comprehensive Prionics-Check group consists of three rapid TSE tests: The Prionics®-Check WESTERN – the most reliable TSE test worldwide, which has given no false positive result in over 20 million tests – the Prionics®-Check LIA – in the well-established ELISA format, especially handy to operate – and the Prionics®-Check PrioSTRIP which will soon be launched and is more compact, faster and easier to handle than any other BSE rapid test yet devised. The benefits: All tests of this system are based on a single sampling procedure and therefore fully compatible, to make up the Prionics®-Check SYSTEM which is suitable for routine analysis of large sample numbers and reliable even under rough, high-throughput conditions.

The implementation of Prionics®-Check is easy and fast due to its convenience and unique handling simplicity. Prionics experts are available to consult and support analytical laboratories, anytime, anywhere. Prionics employs more than 80 highly experienced experts in TSE surveillance solutions – the largest number of scientists in the industry focused on TSE testing and prion research. Since 1997, Prionics has helped to implement successful TSE surveillance programs and Prionics®-Check is used in over 30 national BSE reference laboratories around the world. Prionics' expertise in applied science, BSE surveillance set up, laboratory technician training, laboratory organization and risk communication has made us a valuable asset for the world's cattle industry.

Prionics' world-class research and development team is at the forefront of new developments in prion diagnostics. The company's most powerful advantage is undoubtedly its staff: scientific experts whose pioneering work and acute insights have been recognized by renowned TSE research institutes, generating fruitful scientific collaborations in Zurich and all around the world.

Today it is not possible to determine whether TSEs, like CJD, are transmitted between donor and recipient during blood transfusion. However, a U.K. survey by Llewelyn and colleagues (*Lancet* 2004) has revealed a link between a vCJD death and prior blood donation from a donor who later developed vCJD. Currently there is no test available to detect TSE infective agents in blood. A TSE test for blood samples could certainly help strengthen the current safety measures and to retain patient confidence. At Prionics, research towards development of such tests is in progress.

The Prionics monoclonal antibody 6H4, one of the key components of the Prionics®-Check BSE tests, is used in some of the most renowned prion research studies. Results by the group of Charles Weissmann and Adriano Aguzzi, for example, have shown that the antibody 6H4 might be able to prevent the development of BSE and could maybe even reverse cell damage.

Together with its partners at the University of Zurich, Prionics investigates impulse transmission at the synapses of nerve cells. The team already succeeded in defining intriguing synapse modulating molecules as a target for neurodegenerative disease control. Now Prionics and its research partners at the University of Zurich create the basis for broad screening, to find small molecules which enhance or inhibit the impulse transmission at the synapses.

Olivier Valdenaire (Axovan, Allschwil) presented 'Axovan: A Swiss Biotech Success Story'.

Are we at the end of the 'nuclear winter' that followed the explosion of the technological bubble? The situation seems to improve slightly, especially in the biotech world. We are naturally far from the 2000 euphoria, and probably will not reach these levels for a long time, if ever, but signs are there that should generate a reasonable optimism. This is especially true for Switzerland: the acquisition of Axovan by Actelion (October 2003) and the IPO of Basilea (March 2004) have largely contributed to restore a certain level of confidence, as was shown very recently by the two impressive financing rounds closed by Addex and by Arpida.

The four successful events mentioned above share a common feature: they concern companies mainly dedicated to the discovery and development of small molecules (all of them are at the clinical trial stage). These companies were in addition created and managed by people originating from the pharmaceutical industry. One might think there is nothing to wonder about given the long tradition of Switzerland (especially of the Basel area) in pharmaceutical and chemical industry… This would be forgetting the excellence of Swiss Universities that have also generated a high number of biotech startups. We have to see in this common 'industry' feature the perfect illustration of a very significant mentality and behavior shift of the biotech world, moving from technology-based to compound-based concepts.

A few years ago, a startup had to be based on technology: we were in the '-ics' (genomics, proteomics, *etc.*) golden age where the craziest dreams were made… and sold to pharmaceutical industry and therefore to investors. The situation naturally was not as absurd and extreme as what was seen on the 'dotcom scene', but promises (*e.g.* of a substantial shortening of the time to market) were made that could not be kept: as a consequence investors are now more and more reluctant to bet on purely technological concepts.

The Axovan story nicely illustrates these drastic and recent changes. Axovan was created in April 2000 as a company specialized on orphan G protein-coupled receptors (GPCRs). Our concept was to take advantage of the constitutive activity displayed by GPCRs (when overexpressed and/or mutated at key amino-acid positions) to identify inverse agonists; a special class of antagonists able to lower this constitutive activity. Hits and optimized molecules derived from these inverse agonists would then have been outlicensed to pharmaceutical and biotech companies as part of an information package helping to better understand the function of orphan GPCRs. This technology-driven concept allowed us to close a first round of CHF 8 Mio in June 2000. While validating and implementing this technology, we realized very quickly that the wind was turning: as written above, technology was less and less valued. Thanks to the pharmaceutical industry experience of Axovan's team we could transform within a few months Axovan into an effi-

cient and professional drug discovery platform dedicated to GPCRs (no more orphan GPCRs, but identified, 'validated' ones). In 2003 the achievements were quite impressive: proprietary GPCR-dedicated cheminformatics, a valuable, both GPCR-biased and diverse collection of 60,000 drug-like small molecules, a team of 25 chemists and 15 biologists. Within 30 months, 40 receptors had been screened, three out of five advanced projects had reached preclinical level and five composition patent applications were filed. Clinical Phase I studies for our first 'Axovan-born' molecule were scheduled for 2005.

Looking back, we are still impressed by the quality of Axovan drug discovery and by its achievements. But the truth is that a great part of the value recognized to Axovan by the investors of our B round (CHF 30 Mio) and later by Actelion was linked to something else: clazosentan. Clazosentan (formerly Ro-61-1790), an endothelin receptor antagonist (ET_A specific) discovered in Hoffmann-La Roche was clearly an accelerator and a key factor of the Axovan success story. Like a number of good drugs, clazosentan does not have a straightforward history: outlicensed in 1998 it came back to Roche with a 'failure' reputation two years later, and was probably doomed to stay for ever on the shelves. Our team fortunately knew the compound: some of us had even directly participated to its discovery and characterization during their 'Roche' time. We knew how good and promising the molecule was and were not deterred by what was seen as a recent failure. Axovan in-licensed Ro-61-1790 in April 2002 and could 'restart it', concentrating on the compound's originally foreseen indication: delayed vasospasm following subarachnoid hemorrhage. In October 2002 the first patient was enrolled in a European Phase IIa (34 patients, double blind and placebo-controlled) study. In July 2003 we obtained extremely promising results, some endpoints being achieved with statistical significance in spite of the very low number of patients!

In August we published a short press release, and within two months of accelerated and intensive negotiations, signed a binding termsheet with Actelion, under the terms of which Axovan was acquired for CHF 65 Mio upfront payment and up to CHF 187 additional milestone-linked payments. The deal was unanimously greeted as an excellent one for both parties, and this is still true almost one year after: 90 % of Axovan employees are still working in Actelion, our discovery projects are pursued with more preclinical resources, and clazosentan is being developed with all the experience and critical mass necessary to conduct large-scale clinical trials.

What is the moral of this story? That a biotech nowadays needs to be at least in Phase II to be a success (*i.e.* to provide a profitable 'exit' to its investors)? This is certainly true. But it would be a mistake to consider the presence of a clinical-stage molecule as an investment prerequisite. This is unfortunately more and more the case. As a result many companies are desperately looking for and in-licensing any kind of clinical compound (including the worst ones) only for the sake of survival. This 'rush to clinics' will generate in the coming years a high number of biotech failures (higher than the normal pharmaceutical industry attrition rate) and a lot of disappointment. Clinical development is difficult: you need an experienced team of professionals, but also a real compound… Better indeed to bet on a good preclinical molecule than on a bad clinical one! This seems obvious, but is not always applied… And unfortunately we see more and more absurd situations where a young biotech has outperformed, delivered excellent preclinical molecules but is in financial difficulties. This provides excellent opportunities for the investor who is able to identify such companies.

Sabine Pierau (Morphochem AG, Basel) showed compounds effecting 'Dual Inhibition of NEP (Neutral Endopeptidase) & DPP IV (Dipeptidyl Peptidase IV) as a Potential Treatment for Type 2 Diabetes'.

A significant, rapidly growing fraction of the human population is affected by type 2 diabetes, a disease characterized by elevated blood glucose levels and relative insulin insufficiency. Glucose-dependent insulin secretion is promoted by incretins, predominantly glucose-dependent insulinotropic peptide (GIP) and glucagon-like peptide 1 (GLP-1). The available evidence suggests that enhancement of incretin action may be useful for lowering blood glucose in subjects with type 2 diabetes mellitus [14].

The activity of the potent stimulators of insulin secretion, GLP-1 and GIP, is rapidly abolished by the serine protease dipeptidyl peptidase IV (DPP-IV, CD26, EC 3.4.14.5)-mediated truncation. *In vivo* administration of synthetic inhibitors of DPP-IV prevents N-terminal degradation of GLP-1 and GIP, resulting in higher plasma concentrations of these hormones, increased insulin secretion and, therefore, improved glucose tolerance. This has led to an elevated interest in inhibitors of this enzyme for the treatment of type 2 diabetes. GLP-1 levels fall rapidly following postprandial excursion, and clearance reflects the actions of the kidney, enzymatic inactivation by DPP-IV and neutral endopoeptidase (NEP) 24.11, where NEP is responsible for a fast renal elimination of GLP-1 with a 50–70% extraction [15–17]. An improvement of GLP-1 stability has been shown by a combined inhibition of NEP and DPP-IV in anaesthetized pigs [18]. Deacon and co-workers have shown that treatment of diabetic rats with a combination of a DPP-IV and a NEP inhibitor results in glucose-lowering effects superior to those observed using only a DPP-IV inhibitor.

*Human DPP-IV***:** DPP-IV is a multifunctional type II transmembrane serine protease glycoprotein. Its multiple properties include a highly specific serine protease activity that inactivates or generates biologically active peptides [19][20]. Several classes of DPP-IV inhibitors have been described for this enzyme [21–24]. Among these the 2-cyano-pyrrolidines, which were predicted to form a covalent imidate adduct with the active site serine of DPP-IV [25]. This has recently been shown crystallographically by Oefner *et al*. [26].

*Human NEP***:** Neprilysin (NEP; EC 3.4.24.11), also known as neutral endopeptidase, enkephalinase, CALLA or CD10, is the prototype of the M13 subfamily of a type II integral membrane zinc-dependent endopeptidase. NEP regulates many peptides particularly vasoactive peptides and peptides in the central nervous system. Inhibitors are known as vasorelaxants and analgesics. A number of three-dimensional structures of this zinc-dependent metalloendopeptidase have been determined as complexes with various types of specific inhibitors, giving insight into the zinc ligation and sub-site specificity of the enzyme [27][28].

The Dual NEP/DPP-IV Inhibition Concept for the Treatment of Type 2 Diabetes: Based on the structural knowledge of NEP and DPP-IV, in particular the understanding of their sub-site specificity, a dual inhibition concept can be envisioned. Inhibitors of DPP-IV can be linked *via* their terminal unprimed substituent with NEP inhibitors either with their terminal unprimed residue, their two-primed residue or the C-terminal moiety as shown in Fig. 1. Some potential inhibitors are shown in Fig. 2.

We focused our efforts on combining a phosphinic acid inhibitor of NEP with a cyano-pyrrolidine inhibitor of the DPP-IV enzyme. The linking motive has been deduced from the knowledge of the subsite specificity of both enzymes. The compound MCB 3937 (Fig. 3) inhibits NEP with an IC_{50} of 0.72 μM and DPP IV with an IC_{50} of 0.35 μM. In order to establish the basis for synergy thereby validating the dual inhibition concept pure NEP (MCB 4182) and DPP IV (MCB 4288) in-

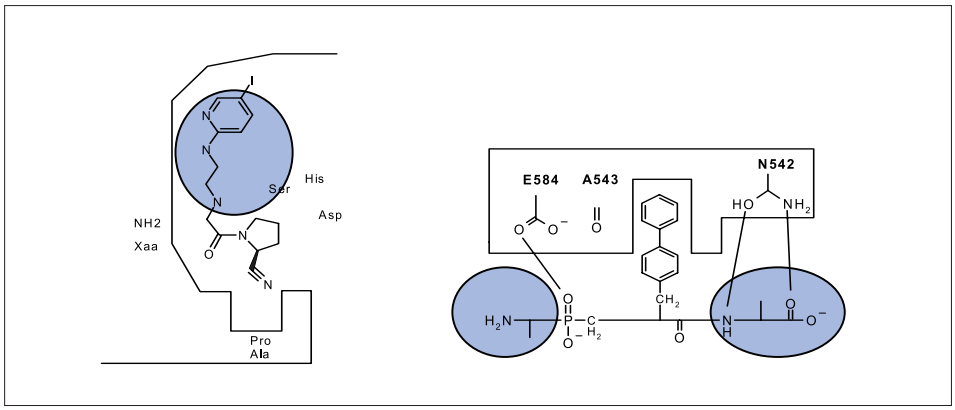


Fig. 1. NEP – DPP-IV substituent variability

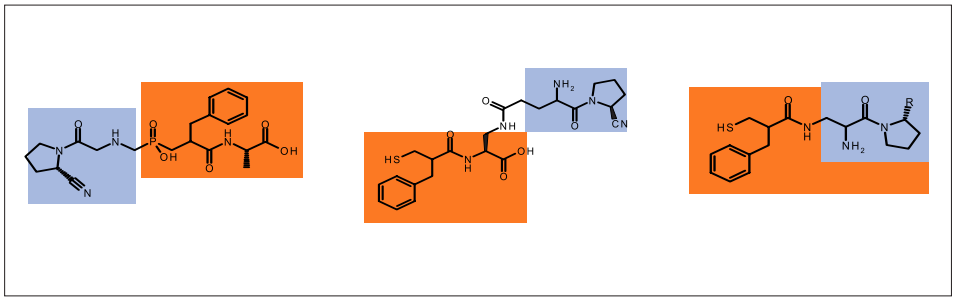


Fig. 2. Example structures of bi-functional NEP – DPP-IV inhibitors. The NEP part is shown in orange, the DPP-IV part is shown in cyan.

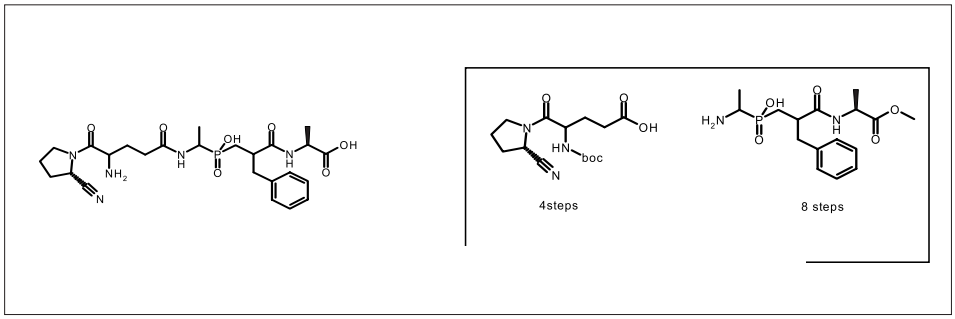


Fig. 3. The dual inhibitor MCB3937 consists of two components

Clinical Development Pipeline

Phase	Product/Compound	Type	Indication
III	Tracleer®	GPCR antagonist	Class II PAH
III	Tracleer®	GPCR antagonist	IPF (Idiopathic Pulmonary Fibrosis)
III	Tracleer®	GPCR antagonist	ILD (Interstitial Lung Disease) in Scleroderma
III	Tracleer®	GPCR antagonist	DU (Digital Ulcers) related to Scleroderma
III	Veletri™	GPCR antagonist	Acute Heart Failure
III	Zavesca®	Substrat reduction therapy	Type 3 Gaucher, Niemann-Pick, Tay-Sachs disease
II	Clazosentan	GPCR antagonist	Vasospasm as consequence of subarachnoid hemorrhage (SAH)
II	Tracleer®	GPCR antagonist	Metastatic Melanoma
II	Veletri™	GPCR antagonist	Hepatorenal Syndrom (HRS)
II	Urotensin-II receptor antagonist	GPCR antagonist	Cardiovascular

Fig. 4. Actelion's clinical development pipeline

hibitors (not shown) have been synthesized. MCB 4288 lacks the cyano functionality while 4182 has an ester instead of the free acid at the C-terminus. A detailed biological characterization of these compounds has been performed.

Having the biological and biostructural systems in place, we could synthesize the first dual inhibitor of NEP and DPP IV and, to the best of our knowledge, the first dual serine-metallo protease inhibitor. The dual inhibitor shows no toxicity, is metabolically stable, orally bioavailable and biologically active *in vivo*.

Morphochem AG is a development stage chemistry and chemo-informatics company with operations in Munich, Germany, and Basel, Switzerland. Morphochem has built a novel drug discovery process based on its proprietary chemistry foundation. The company's multi-disciplinary approach integrates innovative chemistry and chemo-informatics into a seamless, fast and cost-effective process that allows Morphochem to discover proprietary and efficacious small molecules for almost any drug target. Through the application of its drug discovery capabilities, the company has created its in-house pipeline of high-potential small molecules, two of which are currently in preclinical development.

Thomas Weller (Actelion, Allschwil) presented 'Actelion's Drug Discovery Process: Focused Research in Pursuit of Success'.

Founded in late 1997, Actelion Pharmaceuticals Ltd. focuses on discovery, development, and marketing of innovative drugs for significant unmet medical needs. In the meantime, Actelion has become an independent, profitable biopharmaceutical company, with its corporate headquarters located in Allschwil/Basel, Switzerland. Actelion combines the best attributes of biotechnological and pharmaceutical industries, blending biotech innovation, speed, and flexibility in drug development, regulatory affairs, and marketing.

Our strategy combines two major goals. Firstly, we aimed to develop a first product and bring it to market as soon as possible. Secondly, considerable effort went into developing an efficient drug discovery unit with the aim to generate a pipeline of clinical candidates.

Our first drug, Tracleer® (bosentan), a dual endothelin receptor antagonist, is approved as the only oral treatment for pulmonary arterial hypertension (PAH), a chronic, life-threatening disorder which severely compromises the function of the lungs and heart. Our second drug, Zavesca® (miglustat), is a small-molecule oral therapy for the treatment of type 1

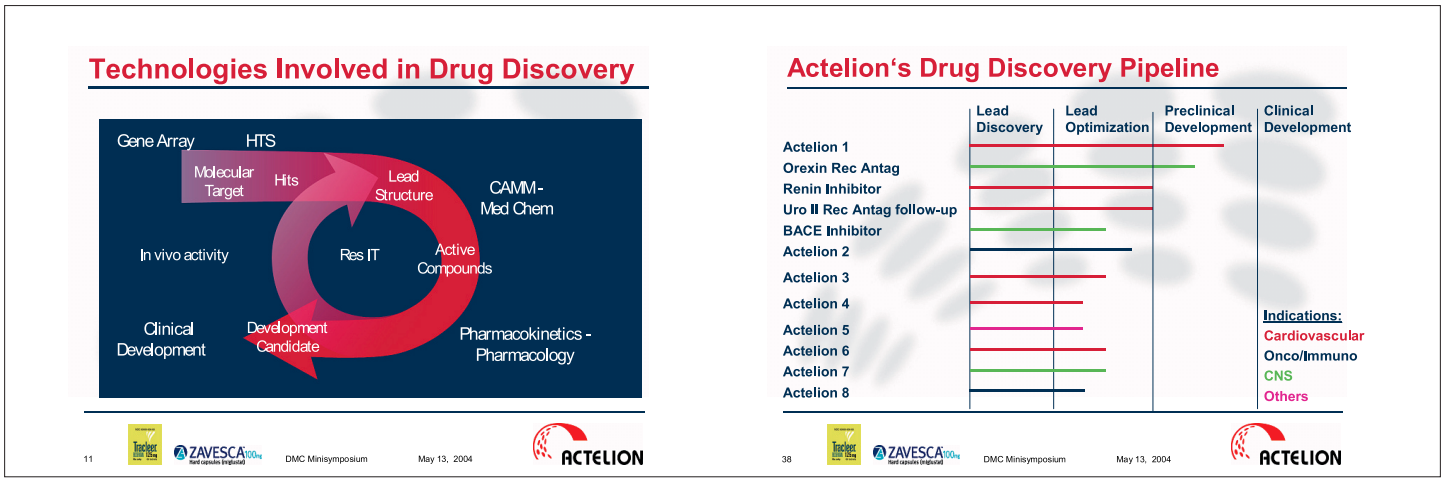


Fig. 5. Technologies involved in drug discovery & Actelion's drug discovery pipeline

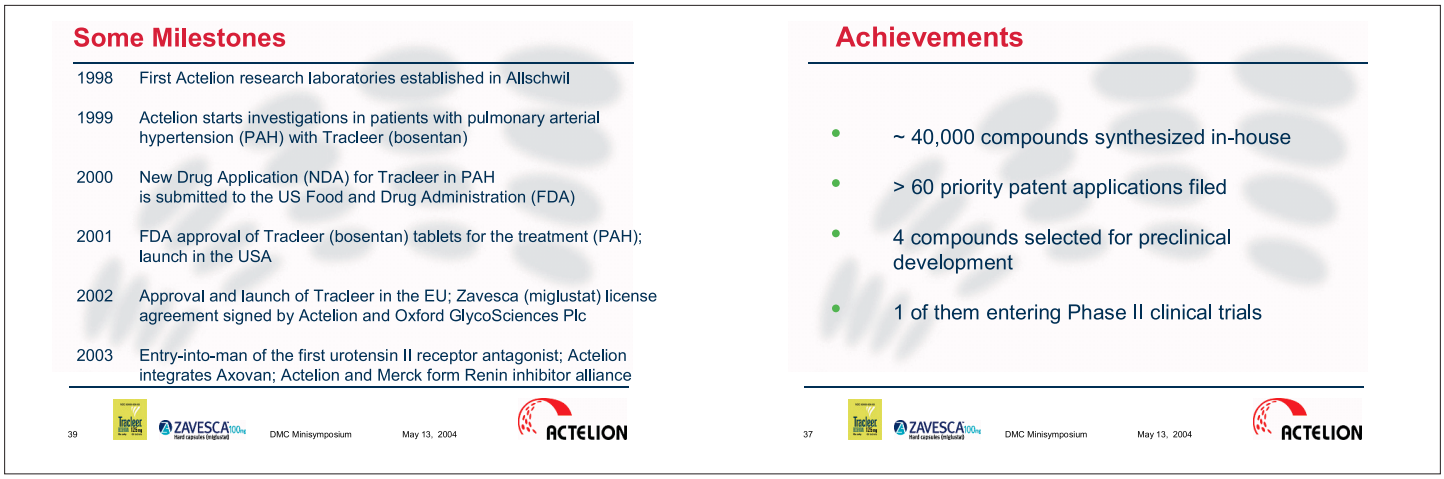


Fig. 6. Actelion's milestones and achievements

Gaucher disease, a genetic lipid storage disorder. Zavesca®, developed by Oxford Glycoscience plc (now Celltech Group), has been licensed by Actelion.

In addition to exploring new indications for Tracleer®, Zavesca®, and two other endothelin receptor antagonists, *i.e.* Veletri™ and clazosentan, we have initiated clinical testing of the first oral urotensin-II receptor antagonist, a new and innovative therapeutic principle for cardiovascular and metabolic diseases (Fig. 4).

Our drug-discovery efforts focus on low-molecular weight molecules. Research projects are based on two molecular platforms to identify potential targets that can be accessed with small molecules, *i.e.* G-protein-coupled receptors (GPCRs) and aspartyl proteases. All these projects have the potential to satisfy important unmet medical needs in cardiovascular, central nervous system, oncological, and immunological indications.

During the past five years, we have succeeded to establish important technologies involved in the various aspects of drug discovery (Fig. 5): gene array techniques in molecular biology

- high-throughput screening of our library comprising 230.000 compounds
- X-ray structure determination of proteins in structural biology
- molecular modeling to support medicinal chemistry as well as parallel synthesis efforts
- handling of large data sets using proprietary software
- *in vitro* as well as *in vivo* pharmacokinetics
- animal models in pharmacology
- assessment of physicochemical properties for pre-formulation studies.

In addition, we established an external network of partners for scale-up and production of drug substance, formulation development of drug product, and toxicological studies.

We consider the combination of essential technologies with the art of medicinal chemistry as the driving force of our drug discovery process. Furthermore, the creation of highly motivated project teams with an 'ownership feeling', short decision-taking pathways, a calculated risk-taking attitude, and the efficient interface management between drug discovery, preclinical development, and clinical development are considered potential success factors. Some achievements and milestones are shown in Fig. 6.

Some aspects of Actelion's drug discovery process have been published in a case study dealing with the search for new endothelin receptor antagonists [29].

However, we also have to keep in mind the statements made by prominent 'drug hunters':

- *Successful drug discovery requires persistence* [30].
- Drug discovery still depends on one key factor: good luck [31].

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