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Small Molecule Drug Discovery in the Fast-Changing World of Biotechnology

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Abstract: Generally referred to as Europe's largest Biotech company, Serono, with global headquarters in Geneva, has long been known for its portfolio of therapeutic proteins, which has, so far, resulted in market approvals of several recombinant products in the areas of reproductive health (Gonal-F, Luveris, Ovidrel), metabolism-endocrinology (Saizen, Serostim), neurology (Rebif), and dermatology (Raptiva). In the late 90s, 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 late 1997, Serono acquired the GBRI (Glaxo Biomedical Research Institute), now SPRI (Serono Pharmaceutical Research Institute), located just outside Geneva, and during the following year therein established a new, state-of-the-art Chemistry Department, with the necessary manpower, expertise, as well as the medicinal, analytical, and combinatorial chemistry equipment, including extensive structure- and ligand-based design capabilities. In conjunction with a somewhat smaller Chemistry Department previously established at Serono's Boston-based research site SRBI (Serono Reproductive Biology Institute), Serono's 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 present article will give an account on some of the work performed to date.

Keywords: Chemokine binders · Discrete Substructural Analysis · GPCR modulators · Kinase inhibitors · Phosphatase inhibitors · Shape Similarity Analysis

Introduction

Protein therapeutics, such as hormones and cytokines, exert their action through specific receptors spanning the cell membrane and transducing the extracellular stimuli to the inside of the cell (Fig. 1). Different superfamilies of membrane receptors are known, including 7-transmembrane spanning G-protein coupled receptors (7TM-GPCRs), receptor tyrosine kinases (RTKs), cytokine receptors, and others, and the receptor usage depends on the nature of the extracellular agent. Receptor activation leads to complex intracellular signaling events, with ingenious mechanisms ensuring a correct transduction and propagation of the signal throughout the cell. One of the most universal ways in which the propagation of the signal is controlled (*i.e.* switched on and off), consists in reversible phosphorylation of given substrates (S) at specific signature sites, mediated by phosphorylating and de-phosphorylating enzymes, kinases and phosphatases, respectively. Based on this – simplified – understanding, a number of potential strategies for pharmacological intervention with small molecules can be identified, including (i) compounds binding to the protein ligand, thereby preventing it from exerting its action, (ii) compounds acting at the level of the receptor, and functioning as agonists or antagonists, and (iii) compounds modulating the intracellular signal transduction by inhibiting kinase or phosphatase activity (Fig. 1). At Serono, projects were initiated following each of those approaches, and some of the results will be presented in the subsequent paragraphs. First, however, since there had been no prior history of small molecule drug discovery at Serono, a compound collection had to be established that was to be maximally enriched with privileged chemotypes prone to produce the desired biological activities on the target classes mentioned above. To achieve this, a number of proprietary computational design methods were developed, and partly implemented in the context of an external collaboration to construct a high-value compound collection for primary screening.

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The Foreplay: Design and Construction of a Compound Collection

The screening facilities at Serono were set up to accommodate a relatively small number of compounds (typically <100'000 per screen). As a consequence, the construction of the compound collection had to rely on the acquisition of small, focused sets of compounds biased towards a particular target or a target class. In addition to the conventional approaches described in the literature, two computational tools developed internally proved to be instrumental in this respect. In the first method, termed Discrete Substructural Analysis (DSA) [1–3], a particular biological activity of a given compound is explained (and predicted) based on statistical association with the presence of two-dimensional molecular fragments. Thus, by applying DSA

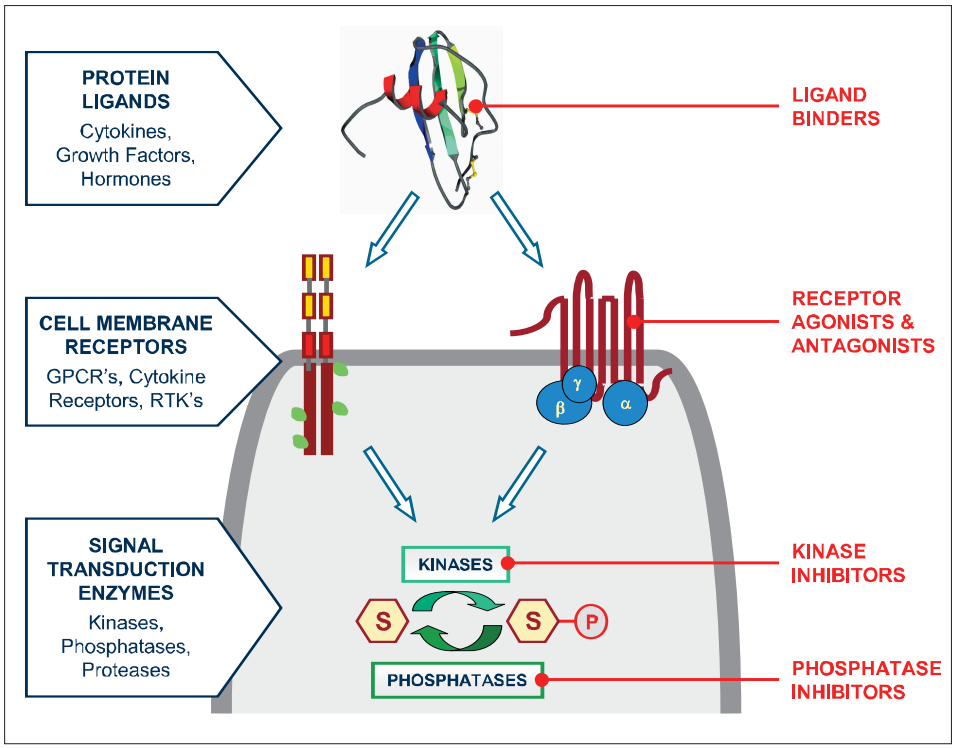

Fig. 1. Potential points of pharmacological intervention directed towards modulating the effects of biologically active proteins.

to a set of compounds known to interact with a given target (or target class), a common 2D-molecular determinant responsible for the biological activity can usually be identified, and subsequently re-used to search databases of commercial compounds and/or to synthesize small combinatorial libraries highly enriched with compounds active against that particular target (or target class). The second computational approach, termed Shape Similarity Analysis (SSA) [4][5], is an attempt to link similar biological activities to similar three-dimensional molecular shapes. Conceptually based on the lock-and-key principle [6], SSA provides an expedient way to generate and visualize the envelope shapes of all compounds present in a given set. Comparing the resulting shape distribution patterns of different compound sets known to interact with closely related biological targets, a significantly higher inter-set similarity was indeed noted in many cases, thus providing another way of selecting compound sets entering a screening campaign. With those two computational methods in hands, a high-value corporate compound collection was put together, containing compounds from commercial sources, as well as proprietary, focused combinatorial libraries from an external collaborator [7]. Importantly, the initial screening hits were found to be tractable starting points conducive to subsequent optimization in medicinal chemistry programs, of which some examples are described in the following paragraphs.

Strategy 1 – Targeting the Ligand: Chemokine Antagonism Revisited

The normal interaction between protein ligands and their endogenous receptor(s) can, in principle, be disrupted by agents binding either to the receptor or to the ligand, thereby preventing them from making a productive contact. Undoubtedly, in a classical Biotech environment, the latter has been (and still is) the first-line approach, whereby the blocking agent is another protein having a certain degree of structural complementarity to the ligand, such as an antibody, a soluble receptor, or a naturally occurring binding protein. In contrast, the small molecule-based research within the pharmaceutical industry has mainly focused its attention on receptor blockade, as illustrated by the fact that today, around 50% of the marketed drugs are modulators (agonists or antagonists) of G-protein coupled receptors (GPCRs). From a purely conceptual standpoint, there is no reason as to why small molecule-based pharmacological intervention should preferably target the receptors, rather than the ligands. Sheer experience has shown, however, that it is straightforward to identify potent and selective agents modulating the activity of GPCRs, which are therefore referred to as a highly 'drugable' class of biological targets. Notably though, this observation is by no means representative of all types of membrane receptors. A telling example is the cytokine receptor family, which has remained highly refractory to the development of small molecule agonists or antagonists, thus rekindling the interest in the alternative strategy of targeting the ligands, rather than the receptors, for pharmacological intervention. And indeed, small molecules have been developed that selectively bind to the cytokine IL-2, and shown to inhibit some of its biological actions [8][9].

In our lab, there has been a long-standing interest in the biology and pharmacology of cytokines, more particularly a subclass thereof, the *chemo*attractant cyto*kines*, or chemokines. In thinking about potential strategies of pharmacological interference with the chemokine network [10][11], we identified chemokine ligand binding as an appealing, albeit not straightforward, alternative to classical receptor antagonism, based on several considerations: first, the field of small molecule chemokine receptor antagonists has become very crowded in recent years [12], as researchers in the pharmaceutical industry have learned how to decipher the structural determinants required for effective receptor antagonism [13]. Secondly, since some chemokines interact with different receptors (and *vice versa*), new inhibition patterns and net biological effects can be achieved by blocking one given chemokine, resembling those of (hypothetical) dual or even triple receptor antagonists. Thirdly, as the biological effects of a chemokine depend on its interacting with both its receptor(s) and the glycosaminoglycan (GAG) chains of cell surface proteoglycans [14][15], a small molecule binder can inhibit the overall process of cell recruitment in several, more specific ways, depending on where it binds on the chemokine surface, and hence cause different biological and pharmacological effects. With this in mind, an NMR-based affinity screen was performed involving the chemokine RANTES and a small set of low molecular weight compounds ('fragments') that had been chosen with a view to maximum functional group diversity and/or proven propensity to pharmacological activity based on the Current Medicinal Chemistry (CMC) database. Gratifyingly, several compounds could be identified that specifically bound to RANTES with dissociation constants in the double-digit micromolar range. Some representatives were subsequently co-crystallized with RANTES and shown to bind in a region adjacent to the GAG binding site (Fig. 2A). In accordance with this finding, those compounds, at high concentrations, were found to be able to displace radioactively labeled RANTES from heparin-coated beads (Fig. 2B). Compound **1** was further evaluated in a mouse model of inflammation and shown to inhibit RANTES-induced recruitment of leukocytes into the peritoneal cavity with an ED_{50}-value of around 3 mg/kg following

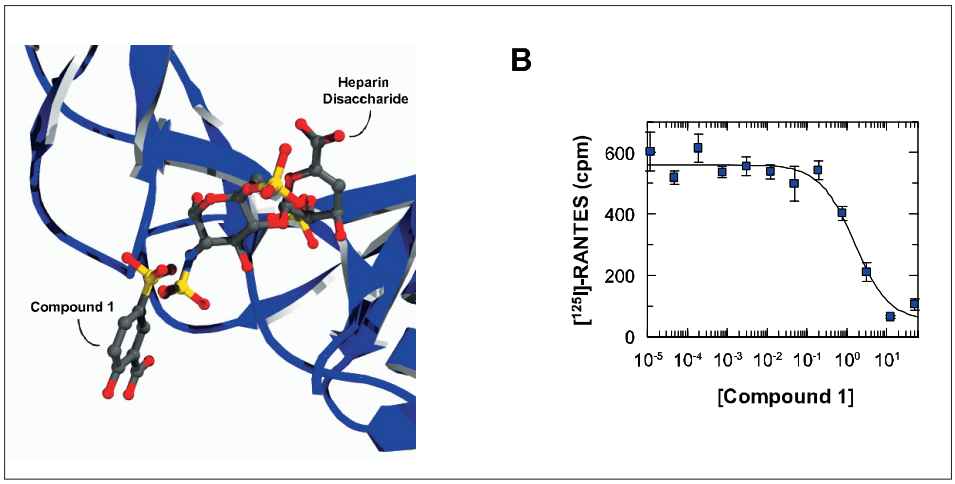

Fig. 2. Small molecule RANTES binders: (**A**) Determination of binding site and binding mode of compound (**1**) by X-ray crystallography. (**B**) Displacement of radioactively labeled RANTES from heparin-coated beads by increasing doses of compound (**1**).

intraperitoneal administration. Attempts to further optimize the binding affinity of the compounds based on the co-crystal structures with RANTES are currently underway.

Strategy 2 – Targeting the Receptor: OT-R Antagonists and FSH-R Agonists

As mentioned above, membrane receptors, in particular those belonging to the superfamily of GPCRs, have proven to be remarkably successful targets for pharmacological intervention. Potent and selective small molecules with appropriate druglike properties have been developed against a wide variety of GPCRs, acting there as antagonists, partial agonist or full agonists. At Serono, with its eminent history in reproductive biology having culminated in several marketed protein therapeutics (*e.g.* Gonal-F, Luveris, Ovidrel), early on, a number of GPCR targets involved in human reproduction were selected for drug discovery programs, two examples of which are described below.

Oxytocin (OT), a nonapeptide containing a cyclic portion formed by a disulfide-bridge, 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. OT exerts its action *via* a member of the GPCR superfamily, termed OT-R, which is expressed in myometrial cells. OT-R is coupled to the $\alpha_{q/11}$ class of G-proteins leading to phospholipase C activation, intracellular synthesis of inositol phosphates and mobilization of calcium. The rise in intracellular calcium concentration promotes a cascade of events including phosphorylation of myosin, that then acts on actin and induces uterine muscle cell contraction. 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 [16]. 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 requiring const. infusion, as well as its poor selectivity towards the closely related vasopressin V1a receptor, limit its use to short-term treatment of the acute phase of preterm labor. 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 [7] led to the identification of the unusual proline-derived oximether compound **2** (Fig. 3), showing good affinity to the human OT-R (K_i = 260 nM), and promising selectivity against the most closely related human vasopressin receptor, hV1a (68% inhibition at 10 μM) [17]. Synthesis of the different stereoisomers of **2** established the (*S*)-configuration at the α-carbon of the proline core to be critical for activity, whilst the configuration at the carbinol center was found to be less important. Separation and NMR-spectroscopic characterization of the double bond isomers of **2** revealed the (*Z*)-isomer to be 3- to 4-fold more potent than the corresponding (*E*)-isomer, which proved to be a general characteristic of this chemical series. Our attempts towards elaborating the SAR around compound **2** initially focused on the oximether moiety, which was considered to be a potential liability due to its instability in strongly acidic medium, as well as its propensity to isomerization. Extensive chemistry efforts produced more than 30 analogues, of which a selection is shown in Fig. 3. Surprisingly, however, all derivatives were found to be considerably less active, pointing to an exquisitely tight SAR around this part of the molecule, which was therefore kept unchanged. The second area of investigation involved the N-substituent of the pyrrolidine ring. Starting from the biphenyl amide motif presented by compound **2**, two first series of analogues were made, in which either the amide or the biphenyl moiety were kept constant, as well as a third series, phenylpiperazine ureas (see Fig. 3). Since, again, none of these derivatives showed any noticeable activity, we decided to adhere to the biphenyl amide moiety and instead explore the effect of introducing substituents on one or both aromatic rings. Again, the SAR proved to be tight, with the 2'- and 4'-positions being the only ones to tolerate small, non-polar substituents. Notably, the introduction of a methyl group at the 2'-position was found to entail a 6-fold improvement in binding affinity, producing the first lead compound, **3** (Fig. 3), which displayed a high affinity toward human and rat oxytocin receptors (K_i/hum = 28 nM, K_i/rat = 82 nM), a 6-fold selectivity against hV1a, and >300-fold against hV1b and hV2. Compound **3** was shown to be active in several animal models of preterm labor, both by intravenous and oral route [18]. One remaining concern with compound **3** was the presence of an amide bond as a potential source of metabolic instability. Consequently, we set out to explore the SAR around the eastern part of the molecule, by generating a series of amide derivatives with a particular focus on tertiary and other amides known to be less prone to hydrolytic cleavage. While none of the newly synthesized amide compounds retained any interesting activity, to our surprise, the corresponding methylester derivative, isolated as a side-product of the solid-supported amide bond forming reaction, was found to be a highly potent OT-R antagonist (K_i = 12 nM), whereas the acid was inactive. Pursuant to this finding, we synthesized a series of different heterocyclic analogues known to behave as bioisosteric replacements of the ester functionality, amongst them 1,2,4-oxadiazole derivatives. Gratifyingly, with those, the binding affinity towards human and rat OT-R was not only retained, but even improved (K_i <10 nM), as was the selectivity against the vasopressin receptors. The absence of the metabolically labile amide bond led to an overall improvement of the pharmacokinetic properties and the oral efficacy *in vivo*. Today, one compound has been advanced to human clinical trials, backed up by several follow-up compounds currently progressing in late-stage preclinical development.

A second GPCR-program, this time directed towards the identification of agonists, was aimed at developing small molecules able to mimic the biological effects of one of Serono's products on the market,

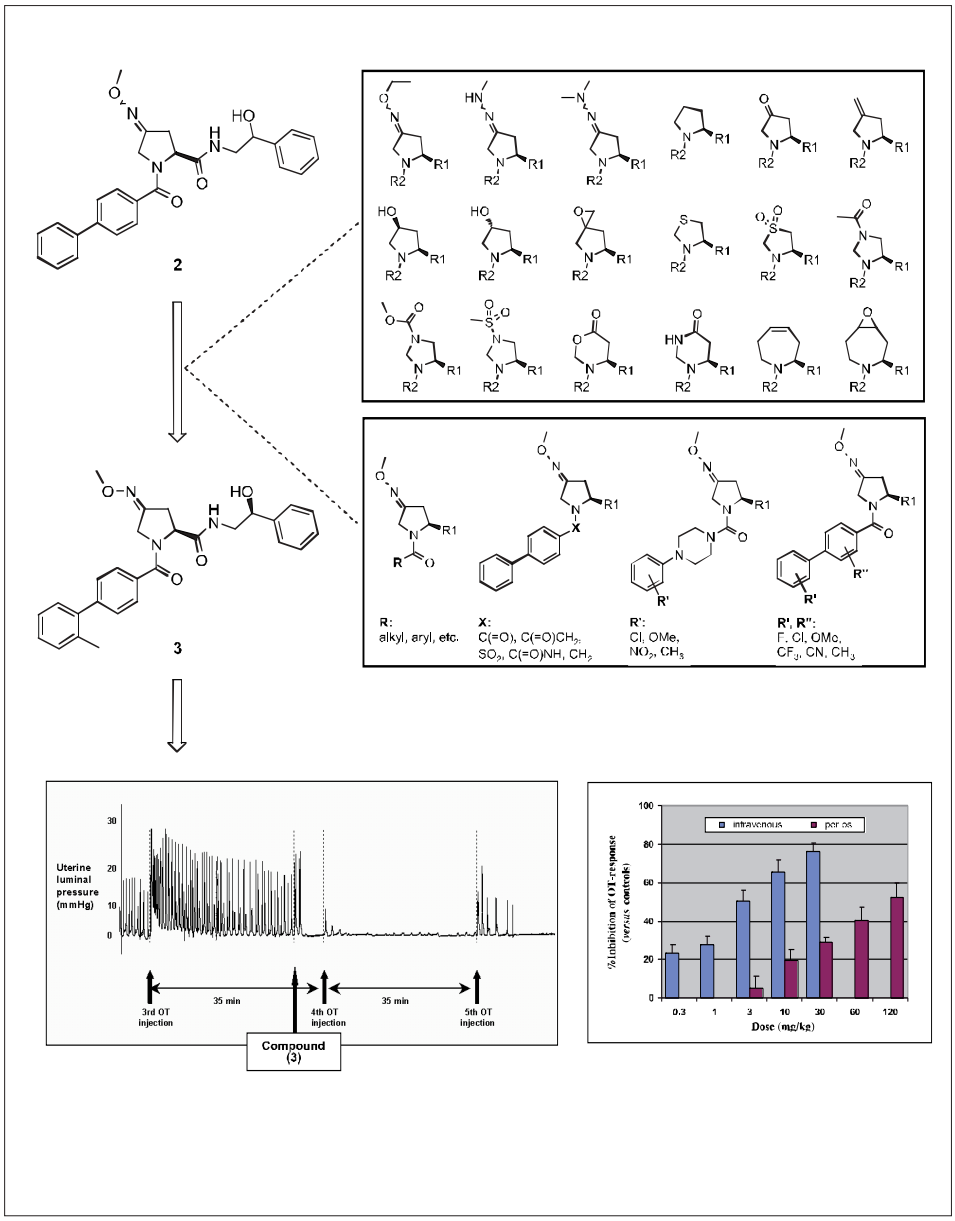

Fig. 3. Development of potent, orally active oxytocin receptor antagonists: dose-dependent inhibition of oxytocin-induced uterine contractions in non-pregnant rats by compound **3** administered by *iv* and oral routes.

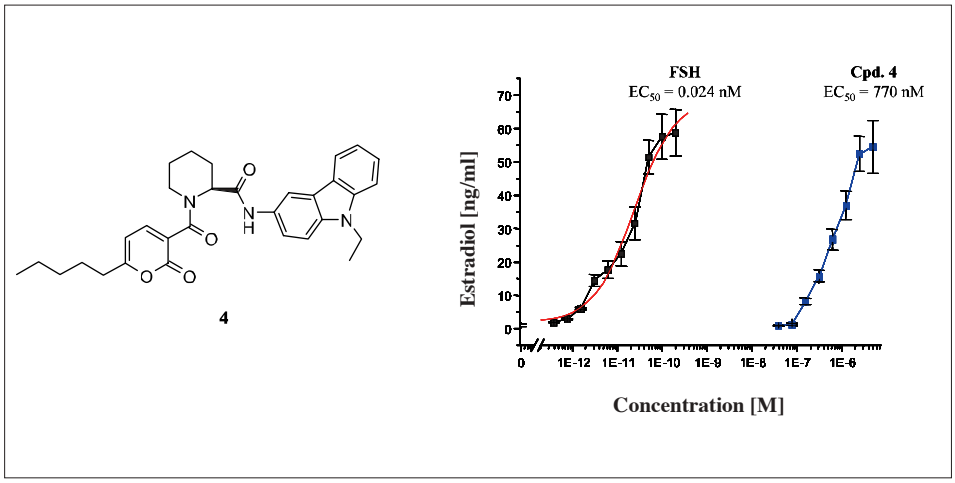

Fig. 4. Development of FSH-R agonists: dose-dependent induction of estradiol production in granulosa cells by compound **4**.

Gonal-F, containing as the active principle human recombinant follicle stimulating hormone (FSH). FSH is a native glycoprotein hormone that is necessary for follicle growth. Its action is mediated *via* a G_s protein-coupled receptor, the FSH receptor (FSHR), present in the membrane of granulosa and theca cells within the follicles of the ovary, where it leads to increased aromatization of androgens to estrogens and, concomitantly, increased proliferation. Decreased levels of FSH result in reduced fertility or infertility, which can, in many cases, be overcome by treatment with Gonal-F. Early on, a project was therefore initiated, with the aim of developing non-peptide, orally active FSH-R agonists displaying similar effects *in vivo* as the native hormone [19][20]. Screening of a combinatorial library enriched with β-turn mimetics led to the identification of pipecolic amides, such as compound **4** (Fig. 4), found to be able to increase the cAMP levels in FSH-R transfected CHO cells with an EC_{50}-value of 4 nM, determined by means of a luciferase reporter gene assay. The effect was shown to be FSH-R specific, since no effect on cAMP levels was noted in CHO cells transfected with genes of two other glycoprotein hormone receptors (LH and TSH receptors), nor in non-transfected cells. Compound **4** was further shown to mimic the effects of FSH in granulosa cells, where it increased the production of estradiol with an EC_{50}-value of 770 nM, paralleled by increased cell proliferation, as measured by ^{3}H-thymidine incorporation. Notably, for FSH itself, an EC_{50}-value of 24 pM (!) was determined in the same assay. Nevertheless, those *in vitro* results proved, for the first time, that it might be possible to mimic the effects of a glycoprotein hormone with a small molecule.

Strategy 3 - Targeting the Signal: Kinase and Phosphatase Inhibitors

Pharmaceutical research aimed at developing agents acting on the outside of the cell has been highly successful in providing new drugs able to modify the course of a wide variety of human diseases. Encouraged by this success, and assisted by an ever-increasing understanding of the intracellular signaling pathways propagating extracellular stimuli on the inside of the cell, a plethora of new drug discovery programs were born, directed towards modulating the activity of intracellular signaling targets, such as kinases and phosphatases. However, whilst the market approval of Novartis' imatinib mesylate (Gleevec), an inhibitor of Bcr-Abl kinase, constitutes a remarkable milestone achievement in this field, it cannot be denied that the development of signal transduction inhibitor drugs, in particu-

lar kinase inhibitors, has met with more difficulties than originally anticipated [21][22]. Amongst the early hurdles encountered along the way are the need for efficient cell membrane penetration, the question about the 'right' selectivity profile against other kinases, and an above-average frequency of occurrence of recurring, idiosyncratic toxicities, arguably associated with the particular chemotype of any ATP-mimetic compound. Notwithstanding this, a sizeable proportion of the small molecule drug discovery efforts at Serono are directed towards the identification of kinase and phosphatase inhibitors, some examples of which are described below.

C-Jun N-terminal Kinase (JNK) has been 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 [23–26]. One compound emerging from these efforts has entered human clinical trials earlier this year, and several back-up molecules are currently progressing in late-stage preclinical development. A detailed account on the JNK program is given in the subsequent article.

The Ras-Raf-MAPK signaling pathway plays a key role in the transmission of mitogenic stimuli to the cell nucleus. Specifically, it links cell surface receptors for a number of cytokines, growth factors as well as several oncogenes, and activated Ras has been implicated in approximately 30% of all human cancers. The kinase MEK, while not implicated as an oncogene product in human malignancies, is a point of convergence in the Ras signaling pathways and is also characterized by high substrate specificity, namely for ERK. Given MEK's eminent role in the Ras signaling pathway, a small molecule inhibitor of MEK has potential to provide a powerful therapeutic for several cancers [27]. Screening of our in-house compound collection against MEK1 revealed an unusual series of potent and highly selective inhibitors, which, of note, did not belong to the subset of the collection designed to have a bias towards kinase targets (see below). Subsequent compound optimization efforts focusing on potency *in vitro*, CYP450 inhibition, and pharmacokinetic properties, particularly metabolic stability, culminated in the identification of compound **5** (structure not disclosed), which compared very favorably to the reference compound, PD184352 (CI-1040), in terms of pharmacokinetic properties and efficacy *in vivo*. Thus, compound **5** was found to inhibit constitutively active MEK1-EE (S218E/S222E) with an IC_{50}-value of 32 nM, and an exquisite selectivity against a panel of 80 kinases. A Lineweaver-Burk analysis indicated a non-competitive mode of action, shedding light on why the chemical series had not been found from the kinase-biased subset of the corporate compound collection. Compound **5** was further shown to inhibit proliferation of C26 cells (a colon carcinoma cell line with an H-Ras GOF* mutation), with an IC_{50}-value of 0.68 μM. Owing to the good overall pharmacokinetic profile of the compound (> 40% oral bioavailability in rodents), this effect translated into excellent oral efficacy in a murine C26 isograft model (Fig. 5), in which statistically significant inhibition of tumor growth was noted at the dose of 30 mg/kg, bid, and even tumor regression at the highest dose of 100 mg/kg. Of note, the reference compound did not show any appreciable activity in the same model. At present, amongst the best compounds belonging to this chemical series candidates are being selected for preclinical development.

Phosphoinositide-3-kinases (PI3Ks) are a family of lipid kinases that can be classified into three subfamilies according to their structure and substrate specificity [28]. Of those, the most extensively studied are the class I PI3Ks, which are present as heterodimers between an adaptor and a catalytic subunit, and are further subdivided into class IA (PI3Kα, PI3Kβ, PI3Kδ) and class IB (PI3Kγ), based on the nature of their subunits. The PI3Kγ isoform, unlike the class IA PI3Ks, operates downstream of GPCRs, where it converts phosphatidylinositol 4,5-bisphosphate, or $PI(4,5)P_2$, into $PI(3,4,5)P_3$, which serves as 2nd messenger for the further propagation of the stimulus inside the cell. The expression of PI3Kγ is mainly restricted to leukocytes, there co-expressed and functionally associated with, *e.g.* chemokine receptors. Inhibition of PI3K is thus thought to represent yet another way to interfere with the chemokine network [11]. 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 [29]. Particularly strong lines of evidence suggest a prime use of PI3Kγ-inhibitors in the treatment of rheumatoid arthritis (RA). With all this in mind, a sizeable PI3K-inhibitor program was established at Serono.

An extensive screening campaign afforded two chemically distinct series of inhibitors with submicromolar IC_{50}-values, presenting different, characteristic selectivity patterns amongst the PI3K-isoforms and towards other kinases. A hallmark achievement was the successful co-crystallization

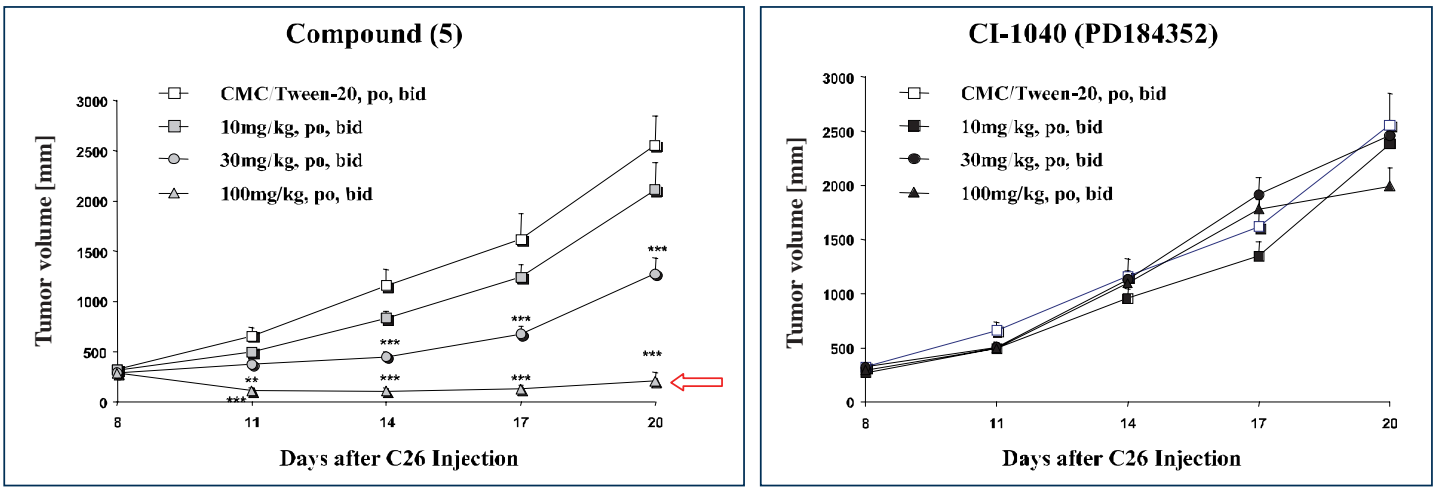

Fig. 5. Development of MEK1-inhibitors: dose-dependent oral efficacy of compound **5** in a murine C26 isograft model, compared to the reference compound CI-1040. Notably, at the highest dose of compound **5**, tumour regression is observed (red arrow).

of representatives of each chemical series with the target PI3Kγ, which proved to be vital for rational compound design and expedient optimization of potency and selectivity. From these efforts, compound **6** (structure not disclosed) emerged as a first milestone. Equally potent on all four recombinant class I PI3K-isoforms (IC_{50} = 2–4 nM), compound **6**, when tested in the monocytic THP-1 cell line, was found to inhibit C5a-induced AKT-phosphorylation (one of the downstream targets of PI3Ks), as well as MCP-1 induced chemotaxis, with IC_{50}-values of 40 nM and 1 μM, respectively. Endowed with excellent pharmacokinetic properties in mice and rats (> 60% oral bioavailability), the compound was further shown to be orally active in several murine proof-of-concept models of inflammation. Current Medicinal Chemistry efforts focus on modulating the PI3K-isoform selectivity of the representatives of both chemical series, in an attempt to determine the most appropriate selectivity pattern for an optimal efficacy *versus* toxicity ratio.

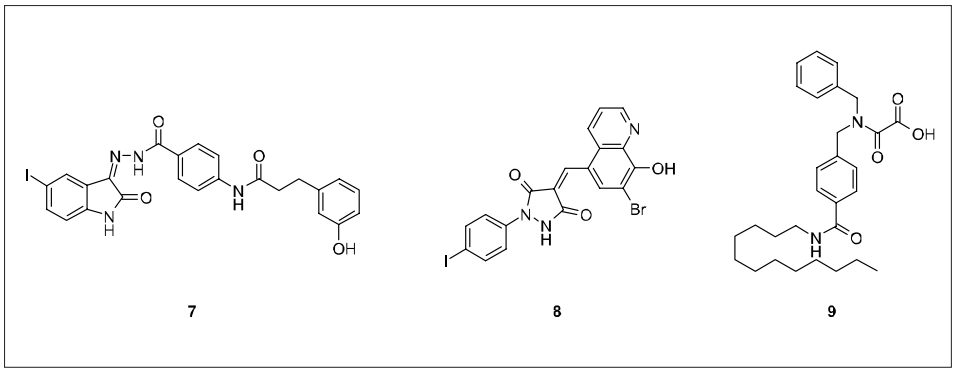

Fig. 6. Development of orally active PTP-1b inhibitors: representative examples of the different chemical series under investigation.

Protein tyrosine phosphatases (PTPs) can be considered as the counter-players of the kinases in cell signaling, and blocking individual PTPs is expected to result in a prolonged activation of specific tyrosine phosphorylation events, thereby sensitizing a cell towards a given hormone or growth factor and hence intensifying their biological effects. Especially for a Biotech company like Serono, whose current revenues are based on protein therapeutics (which include hormones, growth factors, and cytokines), PTPs constitute a promising class of signaling targets, as inhibitors thereof can be expected to intensify and/or prolong the pharmacological effects obtained with the currently marketed products. One particular PTP, PTP-1B emerged four years ago as a new drug target for the treatment of diabetes and, possibly, obesity. The enzyme belongs to a ~90-member gene family of tyrosine phosphatases. Interestingly, this target has very gradually come into focus, following 19th century observations that vanadium salts are of therapeutic utility in diabetes, and the biochemical discovery that vanadate is a potent, non-selective inhibitor of phosphatases. The discovery in 1999, that PTP-1B knockout mice display a phenotype that closely mimics the useful effects of vanadate treatment spurred a vivid interest in PTP-1B as a drug target for diabetes and, possibly, obesity [30]. At Serono, too, a PTP-1B inhibitor program was launched, with the aim of developing potent and selective, orally bioavailable compounds for the treatment of diabetes [31]. Screening of the corporate compound collection afforded two chemical series of submicromolar PTP-1B inhibitors. Structure-based design work in the medicinal chemistry group, based on the concept of the phosphotyrosine mimetic 'warhead', produced another two distinct chemical classes of inhibitors. Some examples of the chemotypes identified are shown in Fig. 6. In order to prioritize the wealth of potent compounds for subsequent PK and pharmacology studies *in vivo*, a combination of calculated properties (particularly the Polar Surface Area, PSA [31]) and measured physicochemical properties (solubility in simulated intestinal fluids) proved to be the most adequate filter for our inhibitors series. Several compounds displayed very favorable pharmacokinetic properties and were shown to be able to reduce postprandial hyperglycemia in obese, diabetic (db/db) mice following single or repeated oral administration. Today, one compound is about to progress to human clinical trials, backed up by several follow-up compounds currently being evaluated in preclinical development.

Conclusion

The different examples described in this paper illustrate the small molecule drug discovery strategy currently being followed at Serono, in the challenging environment generally referred to as the 'post-genomic era'. The way to discover drugs is changing very fast, because new technologies have emerged that have changed the landscape, but also because the time has come, for economical and ethical reasons, to move to the next generation of drugs. The first wave of molecular medicines has successfully been driven by drug discovery efforts targeting almost exclusively proteins that are physiologically modulated, activated, inhibited or recognized by small molecules or peptides. Cholesterol biosynthesis inhibitors, anticancer anti-metabolites targeting DNA synthesis enzymes, or agonists/antagonists of GPCR's activated by biogenic amines (serotonin, histamin, nor-adrenalin, *etc.*) are well-known examples which have led to important new drugs in the last 30 years. During that period of time, progresses in cell biology, molecular biology and structural biology, and more recently in new emerging areas, such as genomics, proteomics or human genetics (in parallel to the human genome sequencing), have identified a large number of new functional proteins as potential drug targets. These proteins do not bind small molecules or peptides in their physiological role, but display their critical function essentially through conformational changes and interactions with other proteins, either inside or outside the cell. The second wave of molecular medicines will focus on this new class of protein targets, and we strongly believe that a biotech research environment, like the one existing at Serono, is best suited to successfully face this challenge. Proteins are at the core of our business, and a deep understanding of protein biology, as well as of the relationships between disease mechanisms and target proteins, are competitive advantages provided by a biotech environment in the race for the discovery of new drugs. In addition, drug discovery is becoming a multidimensional challenge, where understanding structure–activity is not sufficient anymore, where the ability to anticipate and modulate structure–permeability, structure–metabolism, structure–physicochemistry, and structure–toxicity relationships will become a serious competitive advantage. This is not easy, because the molecular basis of these complex properties is not yet well understood and tools available today are far from satisfactory. This is a challenge for the future and we should keep in mind that 'classical' areas like pharmacology, pharmacokinetics and toxicology remain essential pillars for successful drug discovery. Of note, these areas are progressing equally very fast, thanks to genomics and proteomics efforts that will help understand the roles not only of proteins in diseases, but also of proteins and protein networks in metabolism, toxicology or drug transport and biodistribution. In addition,

pharmacogenomics and pharmacogenetics are about to dramatically change the face of drug discovery. It will be the job of the medicinal chemist to understand progresses made in all of these areas and to rapidly apply new findings to the design of better, safer molecules to be developed as medicines ('the right drug, at the right dose, for the right patient, at the right time').

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