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The Hit-to-Lead Process at Schering AG: Strategic Aspects

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Modern organizations conducting drug-discovery programs frequently apply high-throughput screening to generate novel hit structures for the indications of interest. Systematic hit-to-lead processes have been established at most pharmaceutical companies to ensure a smooth conversion of hits into high-quality lead structures. At Schering AG, a hit-to-lead process with distinct selection criteria was introduced in 1999 to identify the best leads possible. The thorough evaluation of hits and lead candidates allows risk assessments, which facilitate the assignment of valuable resources to those lead-discovery projects with the most chances of success. A dedicated hit-to-lead team has been formed to oversee the process and to provide guidance to the respective project teams.

Over the last ten to fifteen years, several new technologies have been introduced into the drug-discovery process in pharmaceutical research. A major impact has been brought about the establishment of high-throughput screening (HTS), which allows the testing of thousands of compounds per day. To take advantage of these modern technologies in drug discovery, the output in chemical synthesis has had to undergo a significant increase through the development of combinatorial chemistry and the generation of large compound libraries, some of which consisted of compound mixtures.

The success rate was initially modest despite the high-throughput approaches in chemistry and biology. At that time, hit follow-up activities were usually conducted within the same project teams that had made the initial identification, and individual experience was the major driving force behind important decisions for lead optimization rather than a defined protocol. Furthermore, target evaluation and validation was not considered to be of high importance. As a consequence, the attrition rate of early HTS projects was extremely high ($>80\,\%$).

As a result of even higher competition in the pharmaceutical industry, it was of utmost importance to increase the chances of success for research projects. Recently, other lead-discovery approaches that exploit natural products, virtual screening, or that simply follow published structures regained importance. However, HTS is still considered the method of choice for the production of lead structures for a large variety of biological targets.[1]

Modern strategies in high-throughput chemistry are focused on quality rather than quantity. Compound libraries are designed to exert lead- or druglike properties and fit-to-target approaches whenever possible. Rigorous purity standards ($>85\,\%$) have been established to avoid the emergence of hits with questionable quality and a low chance of success during follow-up work.[2]

The selection and validation of targets is performed more thoroughly nowadays than ever before. Most companies are strategically creating target-family-oriented project portfolios, which encourage a high degree of cross fertilization between projects. The acceleration of project work was made possible through the introduction of formalized hit-to-lead processes with substantial parallel activities and distinct decision points. Overall, the most important goal of the lead-discovery phase is the generation of lead structures of the highest possible quality. Thus, the hit-to-lead process offers feasibility assessments for drug discovery projects in the early phase, allowing a balance to be reached in the distribution of budget and resources for the costly lead-optimization phase.

At Schering AG, the major factors that influence success rates in lead discovery have been addressed in recent years. Target selection, assessment, and validation are now integral parts of every project started. In addition to considering the biological relevance of a target for a disease to be addressed, the drugability of a target (that is, the ability of a target to be modulated by potent, small, druglike molecules that are often suitable for oral delivery) is intensively evaluated before a project proceeds into the HTS phase. A number of modern assay technologies have been established at Schering AG, allowing work on a large repertoire of different target classes (Figure 1).

Compounds are usually screened in pools of ten at Schering AG. Every test compound is present in two of these pools mixed with different substances. Thus, two positive biological results are necessary to define a compound as a "hit".[3] The pooling strategy was developed in the mid 1990s to save consumables and to perform screening runs quickly. More recently, miniaturization efforts have led to a considerable decrease in costs and in the duration of screening campaigns. Thus, the screening of single compounds has become increasingly popular as data of higher accuracy are being produced and the number of false negatives are minimized. At Schering AG,

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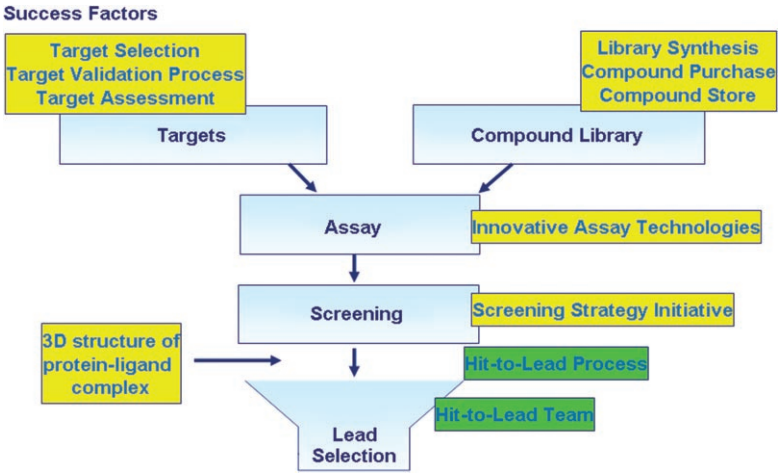

Figure 1. The lead-discovery process.

target-family-oriented libraries (10000 to 20000 members) addressing the major target classes of interest (kinases, G-protein-coupled receptors, proteases, nuclear receptors) and special libraries (natural products, diversity-oriented synthesis libraries) are screened as singles in addition to, or as alternatives to the corporate HTS library.[4] Furthermore, guided screens carried out with interesting primary hits or which are based on results from molecular modeling or virtual screening are gaining importance; this requires a single screening approach and sophisticated compound management.

To cope with the requirements of modern drug discovery, Schering AG has invested heavily in a new compound logistics laboratory, which serves HTS needs on a global basis. A state-of-the-art fully automated compound store that operates with four robotic devices under low humidity at $-20\,^{\circ}C$ is in place, and can retrieve 20000 compounds in 24 h.[5]

Since its introduction at Schering AG in 1999, a standardized hit-to-lead process with defined decision points has played a key role in the success of the company's HTS projects.[6] The overall aim of this process is the generation of high-quality lead structures that serve as optimum starting points for lead optimization. The elaboration of a clear lead-optimization strategy with distinct milestones requires that the liabilities of any lead structure be explored to allow a thorough profiling. Besides defining clear lead criteria, the hit-to-lead process includes the parallel assessment of physicochemical, pharmacokinetic, and toxicological properties, which have not been evaluated at such an early project phase before. Previously, the potency of a compound was the main criterion for selection.

The medicinal chemistry department at Schering AG is heavily involved in the hit-to-lead process, particularly in compound selection activities, in the evaluation of chemical tractability, in the optimization potential of hit substances, and, of course, in the synthetic work related to the hit-to-lead projects. Similar processes and criteria have been introduced at other pharmaceutical companies as well.[7]

A dedicated hit-to-lead team was formed to oversee the process. This team provides guidance for the project teams in terms of building up substantial expertise in the filtering of large hit numbers and in the identification and generation of suitable lead structures. An increasing number of computational tools (prediction of physicochemical properties, docking to a certain target) have recently come into use for support of this process, which is continuously being optimized. The assessment of the quality of a lead structure is carried out by considering the lead structure criteria specific to Schering AG (Table 1).

A suitable lead compound should exert molecular properties in accordance with the Lipinski rules if oral administration is desired, as is the case for the majority of targets at Schering AG. The potency of a lead should be reasonable ($<1\,\mu M$) and should have efficacy in a cellular system. Moreover, a lead should display a certain level of selectivity, which, of course, is very target-specific (usually >10-fold). In contrast to the traditional paradigm of identifying the most potent lead structures possible, it is nowadays much more desirable to identify a lead that exerts a satisfactory overall profile, even if the potency itself is moderate. Pharmacokinetic properties are considered especially important. A lead compound should be cell-permeable and should display sufficient stability in microsomes of various species including human ($>50\,\%$ intact after 30 min). Plasma clearance, volume of distribution, and oral bioavailability in rats should be reasonably high ($>25\,\%$). In vivo proof-of-concept is desirable if the indication allows meaningful studies with non-optimized compounds. The structural class should be synthetically accessible, and modifications should be possible with an appropriate level of synthetic effort. Preferentially, parallel synthesis should be possible for optimization work. A lead should not consist of a single chemical entity, but rather a clus-

Table 1. Lead structure criteria at Schering AG.

- Suitable molecular properties
  - Molecular weight: 200–500
  - $\log P/\log D$: -1–5
  - H-bond donors: 0–5
  - H-bond acceptors: <10
  - Solubility in water: $>5\ mg\,L^{-1}$ (or $10\times IC_{50}$)
- Favorable pharmacodynamics
  - Potency: $IC_{50}=100$–1000 nM
  - Efficacy: Active in cell-based assay
  - Selectivity: >10-fold (project-specific)
- Acceptable pharmacokinetic properties
  - Permeability in Caco2 cells: $>100\ cm\,s^{-1}\times10^{-7}$
  - Stability in microsomes (mouse, rat, human): 50–80 %$R_{30\ min}$
  - In vivo (rat):
    Plasma clearance: $<50\ mL\,min^{-1}\,kg^{-1}$
    Distribution volume: 1–$10\ L\,kg^{-1}$
    Oral bioavailability: $>25\,\%$
- Chemical optimization potential
  - Accessibility
  - Possibilities for modifications (preferably by parallel synthesis)
  - SAR available (analogue generation)
- Patentability
  - Clear patent strategy

ter of compounds. Ideally, active analogues should be present to provide initial structure–activity relationship (SAR) information. Finally, the structural series of the lead must allow the filing of patents for Schering AG. Thus, at least a strategy to generate patentable derivatives of the lead should be developed in this early stage, as the lead itself might belong to a structural class from the public domain. It is quite clear that a lead will not perform optimally against all criteria mentioned. However, as indicated above, a profile that provides information on the particular liabilities of a lead structure should be the end point of the hit-to-lead process, to permit focused and effective optimization in the follow-up efforts. In practice, weaknesses in most of the categories could be overcome, but it is quite clear that a lead compound should not violate all of the criteria at the same time.

Thus far at Schering AG, pool-10 screening has been performed frequently, making use of the corporate HTS library, which consists of 700 000 compounds (Figure 2). To eliminate the false positives that arise through the additive effects of several compounds in a well, all hits are retested as individual compounds for confirmation. The target-family-oriented libraries are screened as singles only to provide additional hits. All confirmed hits are tested at multiple doses. Several hundred 'IC_{50} hits', which interact in a dose-dependent manner with the target are usually identified, marking the entry point of the hit-to-lead process (t_0).

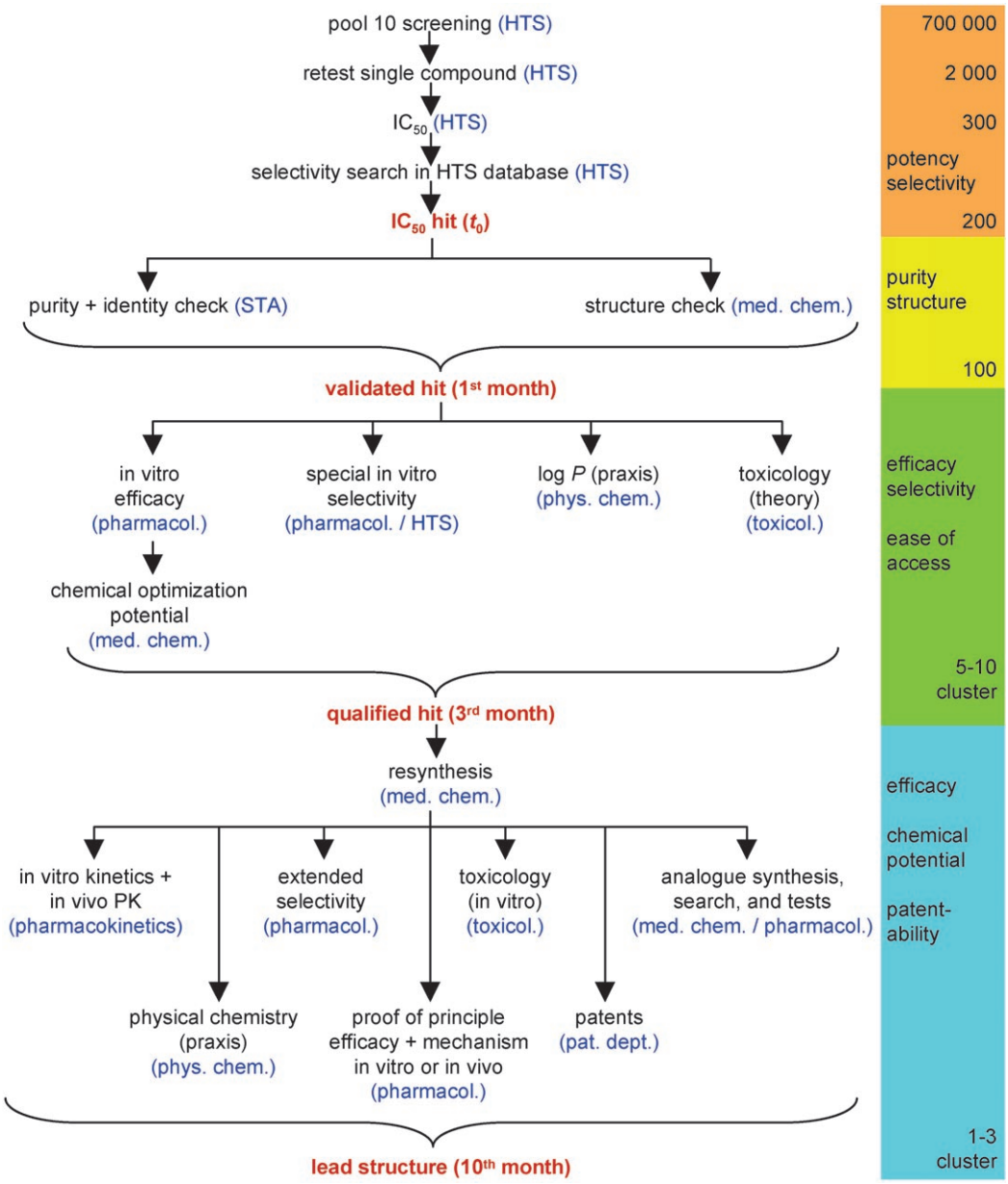

Figure 2. The hit-to-lead process timeline. Departments are indicated with blue text; the colored column on the right highlights the four main phases of the process, with the number of candidate compounds shown. STA = structural analysis department.

Such 'IC_{50} hits' are subject to purity and identity checks, which are routinely performed with HPLC–MS to determine whether the compounds have partly or completely degraded during storage. As strict entry criteria for library compounds are present nowadays in terms of purity and structural quality, the attrition rate at this stage is much lower than it was several years ago. Almost every pharmaceutical company applies purification procedures to all library compounds synthesized[8] to avoid a high proportion of false-positive or false-negative hits. The principal suitability of the structures for further follow-up work is evaluated in parallel from a medicinal chemistry point of view. For cases in which interesting structures fail the structure check, other methods are applied (HPLC with different detection methods and NMR spectroscopy) to examine the limit of the generic high-throughput HPLC–MS technology.[9] Frequently, compounds can be rescued by this process, such as compounds without a UV chromophore or those with low ionization potential in MS.

Compounds which successfully pass the structure checks are termed 'validated hits' ($t = 1$ month), which are tested in a second in vitro assay. Usually, if the first HTS assay is an enzyme-based test, the secondary assay involves a whole-cell system. Initially, the screening of a rather large number of compounds had created a bottleneck, as the biological assays at the early stage of a project did not allow sufficient throughput. This problem could be solved by the application of medium-throughput screening (MTS) technology in all pharmacological departments. The failure of a particular compound or cluster of compounds does not necessarily exclude it from the downstream process, as some library compounds could possess structural features that are not favorable for cell penetration. Furthermore, many 'validated hits' are weakly active in enzyme-based assays ($IC_{50} = 1$–10 μM). Therefore, a clear-cut biological activity in the more complex cellular environment cannot necessarily be expected. In practice, suspicious compounds are investigated in pharmacokinetic assays (Caco-2 cell line) at this stage of the project to minimize the loss of valuable structures. Selectivity assays are performed in parallel to the secondary in vitro evaluation (for example, the in-house panel of 12 kinases), the lipophilicity is investigated (log *P*), and the toxicity is assessed in silico (DEREK score).[10] These data are useful for the overall characterization of a given 'validated hit'. The major criterion for proceeding with a compound besides biological activity in the secondary in vitro assay (occasionally backed up by pharmacokinetic data) is the thorough evaluation of the chemical optimization potential, leading to 'qualified hits' ($t = 3$ months). Although the applicability of parallel or automated synthesis is desired, it is not a prerequisite for further consideration of a structural series. Complex chemistry can be realized even in this early project stage. At this point, clusters of compounds are preferred; singletons, however, are not excluded.

The last phase of the hit-to-lead process should result in high-quality lead compounds. Thus, the evaluation of the 'qualified hits' has to define the profile of the resulting leads. For these investigations, an quantity of ≈ 100 mg of each 'qualified hit' is required. As the corporate compound library accommodates an average quantity of 10 mg of each compound, re-synthesis or purchase of the 'qualified hits' is necessary. In large clusters of 'qualified hits', a number of representatives must be selected for synthesis, as the capacity in the medicinal chemistry department is somewhat limited.

A substantial number of activities are carried out in parallel. In the final stage of the hit-to-lead process, pharmacokinetic behavior (permeability, metabolic stability, inhibition of P450 isoenzymes, plasma protein binding, and in vivo pharmacokinetics) and physicochemical properties (aqueous solubility, pK_a, and stability in simulated gastric fluid) are investigated. Sometimes extended selectivity assessments are executed, such as an external kinase panel. Importantly, the principle mechanism of action is explored under suitable in vitro or in vivo settings, depending on the target indication. Furthermore, toxicity is examined (cellular toxicity and mutagenicity). Patentability of the respective lead candidate series is explored in cooperation with patent professionals to define the 'freedom-to-operate' boundaries. To generate early SAR, analogue generation is carried out by the medicinal chemistry department. As mentioned above, certain libraries can be built around singletons, giving rise to promising new clusters (lead generation).

On average, between one and three lead-structure series are identified in successful hit-to-lead projects by the filtering process, which takes the defined decision points into consideration (Table 2) and applies the lead-generation activities described above ($t = 10$–12 months).

Table 2. Decision points in the hit-to-lead process at Schering AG.

- IC_{50} hit: Dose-dependent potency in the HTS assay
- Validated hit: Identity and purity proven; suitable structure
- Qualified hit: Tested in cell-based assay; evaluated selectivity in vitro; suitable physicochemical (log *P* calculated and measured) and toxicological (calculated) properties; potential for chemical optimization
- Lead structure: Active in relevant cell-based assay; suitable physicochemical properties (measured); suitable pharmacokinetic properties in vitro and in vivo; efficacy in vivo or in appropriate secondary in vitro model to reflect on the potential mechanism of action; clear patent strategy; preliminary SAR; stringent optimization strategy

The hit-to-lead team has played an important role in the implementation of the hit-to-lead process, which began several years ago. Crucial tasks for the hit-to-lead team include providing guidance for the project teams in the process and guaranteeing the capacities of the departments of medicinal chemistry, analytical chemistry, physicochemistry, pharmacokinetics, computational chemistry, and toxicology. Thus, experts from these disciplines are needed in the hit-to-lead process; they are either members of the team or are closely associated. The hit-to-lead team monitors all projects and gives recommendations to the project teams (hit selection, advice to continue or terminate a project). However, the final decisions are made by the sponsoring Corporate Research Business Area (CRBA) at Schering AG which represents the indications (gynecology and andrology, oncology, inflammation, immunology, and diagnostic imaging). The consideration of the hit situation of a particu-

lar project allows the estimation of its feasibility and provides valuable information for the CRBA to assemble their strategic portfolios based on profound risk assessments.

Another important task for the hit-to-lead team is the continuous improvement of the hit-to-lead process. This includes the introduction of new assays for the assessment of pharmacokinetic or toxicological properties such as the unintentional inhibition of the cardiac potassium channel hERG (hERG liability), the evaluation and implementation of software tools for property predictions (most importantly for solubility in water and DMSO), the adaptation of the process for new areas of indications such as diagnostic imaging, and the benchmarking of processes at other companies. Finally, the hit sources are analyzed on a regular basis to collect data that might be useful for the further development of the corporate HTS compound library or target-family-oriented libraries.

As outlined above, expertise and capabilities of the medicinal chemistry department are essential for several phases of the hit-to-lead process. Therefore, an "early chemistry" team has been installed as an important subgroup of the hit-to-lead team. It consists of four chemistry groups (one PhD-level chemist and two technicians each). An "early chemist" is associated with every hit-to-lead project, and is held responsible for providing the necessary information to the project group (there is a maximum of three projects allocated per early chemist). The laboratory capacity from the early chemistry pool is very flexible, and can adjust according to the needs of the project (synthesis of qualified hits and analogues), as the hit-to-lead workflow can jump to high-capacity requirements on short notice. Therefore, the groups in the early chemistry team have to deal with a large variety of different chemistries in short time intervals to overcome these bottlenecks. It has turned out that substantial early chemistry know-how evolves over time which is very valuable for giving well-reasoned recommendations to the project teams. For broadening this know-how in the medicinal chemistry department at Schering AG, an exchange of groups between hit-to-lead and lead-optimization work is planned to take place every two to three years.

Quite recently a group from the automated medicinal chemistry department was integrated into the early chemistry team to extend the lead-generation activities. Instead of synthesizing a small number of analogues around a 'qualified hit', the production of libraries of up to 300 compounds is now possible. The time frame for that approach depends critically on the availability of scaffolds (produced in the early chemistry team or elsewhere in the medicinal chemistry department) and building blocks (extension of the Schering AG building block collection is ongoing). In an average project, two or three quick optimization cycles are possible, giving a more solid data set for lead selection.

The processes of selecting 'validated hits' or 'qualified hits' usually involves all early chemists taking advantage of individual experiences, minimizing biased decisions. Aside of this, the early chemistry team is responsible for the synthesis of reference and tool compounds and also supports virtual screens (through the purchase or synthesis of virtual hits).

Initially, certain conflicts occurred when critical recommendations of the hit-to-lead team (for example, to wind down a project) were questioned by the CRBAs. However, experiences gathered throughout the hit-to-lead process by the hit-to-lead team are now very well-appreciated and highly accepted by the CRBAs.

In a kinase project, the corporate HTS library (at that time 500 000 compounds) was screened by using the pool-10 setting (Figure 3). After confirming 2000 hits at 10 μM, dose-dependent testing in the enzyme-based assay delivered 162 'IC_{50} hits', which were subjected to the validation process. As the corporate HTS library still contained a significant portion of compounds of modest purity at that time, only 80 'validated hits' were nominated. This reflects the high attrition at this stage with the old HTS library, which has been completely renovated in recent years. Qualification was carried out after data regarding cellular activity and a thorough evaluation of the chemical optimization potential were available. Information on physicochemical, pharmacokinetic, and toxicological properties was used to provide a ranking of the 'qualified hits', which came to a total number of 13, including several clusters and singletons. Two clusters were investigated with high priority, as the structural motifs were rather new in the kinase arena at that time. Thus, the probability of gaining access to novel intellectual property for Schering AG was very high. After extensive evaluations of the 'qualified hits', and more than 40 synthesized analogues (which required significant capacity in the medicinal chemistry department), these clusters were promoted to lead series roughly 12 months after completing the HTS.

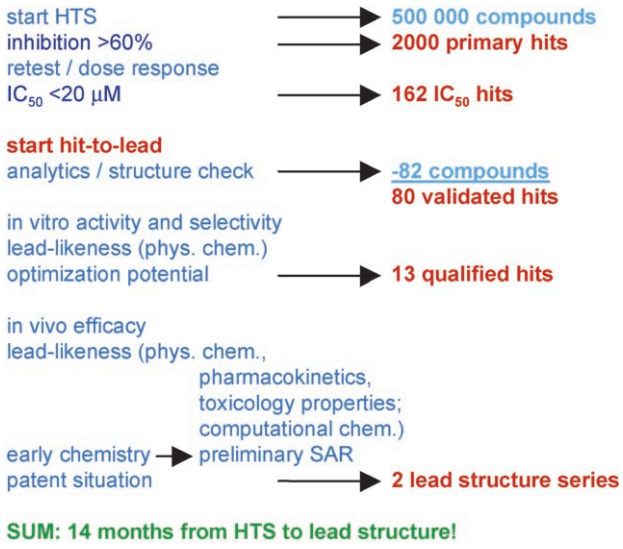

Figure 3. The hit-to-lead selection of a high-quality lead compound; the example process shown is for kinase inhibitors (anticancer target).

In all, several dozen projects have passed the hit-to-lead process at Schering AG, with the attrition rate now at approximately 60%. The hit-to-lead process has proven to be a valuable tool to assess the feasibility of early projects and to provide solid ground for making decisions prior to the lead-optimiza-

tion phase, which requires substantial resources. The rigorous filtering process together with the possibility of elaborating effective lead-generation strategies (focused on optimization of potency or on the improvement of physicochemical and pharmacokinetic properties) delivers high-quality lead structures in a considerable number of projects.

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