Chapter 7

High-Throughput Screening and Drug Discovery

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Ever tried Ever failed. No matter. Try again. Fail again. Fail better.

Samuel Beckett, "Worstward Ho"

I. INTRODUCTION

Until about 1980, information on drug targets at the molecular level was scarce and drug discovery was mostly driven by data obtained from testing relatively small numbers of compounds in pharmacological models.[1–4] Marketed drugs generally originated from lead structures that had well-defined medicinal properties, such as natural products or other drugs. In 1988, for example, Kurt Freter noted that all of the new drugs that had been approved by the Food and Drug Administration (FDA) in 1985 appeared to be the result of analog-based approaches.[5] The number of exploited lead structures was relatively small: Walter Sneader categorized some 244 drug prototypes, fewer than 140 of which would be considered drug-like as currently defined and, among these, only 25 originated from screening processes.[6] Screening, either random or directed, was a low throughput, manually intensive process generally conducted in animal models and usually directed toward the identification of drug rather than lead candidates.

Even through the end of the 1980s a screening capacity of hundreds of samples per week was deemed high-throughput. Over the past 20 years drug discovery has moved to a target-based focus[7] that has been enabled by advances in molecular biology, automation, combinatorial chemistry, and informatics. Many thousands of compounds can now be screened rapidly against a biological molecular target or cellular process and, in most drug discovery organizations, high-throughput screening (HTS) or ultra-high-throughput screening (uHTS) is a central paradigm for the identification of novel lead structures. Although HTS approaches are now also applied during lead optimization (LO) to the assessment of properties such as solubility and cytochrome P450 inhibition, the focus here is on impact related to lead discovery rather than LO.

II. HISTORICAL BACKGROUND

Over the years, various screening strategies have been applied to the identification of drug and lead candidates. The screening of natural product extracts for bioactivity followed by the isolation of the active principle or principles,

which may be classified as the screening of compound mixtures against multiple biological targets simultaneously, has been a highly productive source of drug and lead discovery. The identification of cyclosporine A as an effective immunesupressant is illustrative of this process.[8] Screening of a fungal extract for antimicrobial activity led to the isolation of a number of cyclosporins that were subsequently found to possess immunosuppressive properties. The molecular target mediating the pharmacological activity was only identified several years after marketing approval was obtained for the drug.[9]

Screening of discrete synthetic compounds *in vivo* or *in vitro* for a targeted pharmacological or phenotypic effect, essentially the testing of individual compounds against multiple targets simultaneously, constitutes a second approach. This approach was employed by Ehrlich in the early part of the last century to identify arsphenamine, an important early anti-infective drug.[10] From the 1950s onward, the National Cancer Institute has screened many thousands of samples per year *in vivo* and *in vitro*[11] and identified anticancer agents such as hydroxyurea[12] and the lead structure for carmustine[13] using this approach.

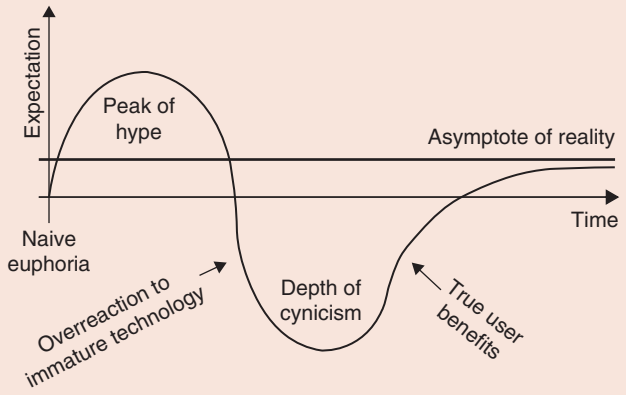
A third approach, screening large numbers of individual compounds or defined pools of compounds against discrete biological targets has been effectively enabled in recent years and constitutes a core concept in HTS. Although there are certainly historical examples of LO campaigns driven by test data derived against isolated targets,[14,15] the capacity for such testing in a high-throughput manner to identify novel lead structures was previously limited by the relatively small numbers of synthetic compounds available for screening and the lack of well-characterized biological targets. Advances in molecular biology provided access to many potential drug targets as pure or overexpressed proteins and made them available for molecular or cellular assays. Improved automation and informatics provided tools for the organization of screening libraries and the collection and interpretation of the large quantities of data generated by screening campaigns. Finally, combinatorial chemistry and high-throughput synthesis methods provided large collections of compounds to fuel the screening process. In all, the combined application of these new technologies has enabled a HTS approach to lead identification, and now large numbers of discrete small molecules can be assessed for activity against a well-defined biological target within a relatively short period of time.

The widespread adoption of HTS and associated technologies for lead identification led the expectation that an increased number of drug candidates would progress through the clinic; however, it is only relatively recently that the positive impact of HTS on drug discovery has become apparent. As shown in Table 7.1, from 2005 onward a significant number of approved small molecule drugs has emerged from screening leads, whereas in the decade before, one or two new drugs per year at most originated from any screening approach. One key reason for this time-lag or delay in impact resides in the long timelines of the modern drug discovery process, often 12 years or more from project initiation to drug approval. However, also contributing to this delayed impact is the time required to develop processes that effectively leverage the application of these new technologies to lead identification, in other words – a learning curve (Box 7.1). For instance, Chris Lipinski and colleagues noted in 1997 that HTS campaigns tended to produce relatively large, lipophilic lead molecules.[16,17] Since it is common during LO to enhance potency through the addition of lipophilic substituents, many discovery campaigns based on screening leads provided large, insoluble lipophilic drug candidates-molecules difficult to progress successfully through the clinic.[18] The resulting "Rule of 5" provided a correlation between molecular properties (MW, Log *P*, number of

TABLE 7.1 Drugs Derived from Screening Leads

Drug	Approved	Lead source
Nevirapine[73]	1996	Corporate historical
Delavirdine[74]	1997	Corporate historical
Efavirenz[75]	1998	Corporate historical
Tirofiban[76]	1998	Directed screening
Bosentan[58]	2001	Corporate historical
Gefitinib[77]	2002	Computational screening
Sivelestat[78]	2002	Corporate historical
Aprepitant[79]	2003	Different company
Cinacalcet[80]	2004	Drug
Sorafenib[81]	2005	Commercial acquisition
Tipranavir[82]	2005	Drug
Conivaptan[83,84]	2005	Different company
Mozavaptan[85]	2006	
Sunitinib[86]	2006	
Dasatinib[87]	2006	Target switch
Sitaxentan[49,50,51,52]	2006	Drug/directed screening
Sitagliptin[88]	2006	
Ambrisentan[60]	2007	Agrochemical
Maraviroc[89]	2007	

BOX 7.1 The Evolution of New Technologies



Source: James C. Bezdek: Fuzzy models – What are they and why? *IEEE Trans. Fuzzy Syst*. 1993, 1. © 1993 IEEE, Reprinted with permission.

H-bond donors and acceptors) and the potential to achieve physical properties consistent with acceptable oral absorption, and spurred many subsequent efforts to qualify drug-like molecular properties. Further refinements, based on analyses of lead and corresponding drug molecules, led to the proposal of distinct lead-like molecular properties (e.g. MW < 300, Log $P < 3$)[19–21] and assessments of drug and lead-like characteristics are now a routine part of the progression from screening-hit to lead series. Almost simultaneously, also in response to the challenges associated with identifying high quality lead structures from screening campaigns, a formalized "hit-to-lead" process distinct from LO emerged.[22] Today, in most large pharmaceutical organizations, dedicated teams are responsible for the progression of screening hits to the start of LO.

III. FROM SCREEN TO LEAD

Fundamentally, the quality of the lead structures obtained from screening will depend on the nature of the compounds in the screening collection, the quality of the assay system, and the processes that are in place to progress from the assessment of active samples to the delivery of a lead series.[23]

A. Compound collections

Corporate screening collections now often exceed one million compounds.[24] Ideas on the optimal size for a collection range from the suggestion that two to three million suitable compounds should deliver multiple starting points from any given screen[25] to an estimate that up to 24 million compounds would be needed to ensure potent hits for all targets.[26] Collections consist of varying ratios of compounds originating from a number of sources such as previous drug discovery campaigns, combinatorial or high-speed chemistry, and acquisition or purchase from academic or commercial vendors. Natural products of plant, animal or microbial origin, either pure materials or extracts or mixtures, are also frequently part of a screening collection.

Compounds derived from previous drug discovery campaigns, historical or heritage compounds, may lack structural diversity particularly if prior research was focused in specific, limited target areas. Recent analyses of screening collections, prompted by mergers and consolidation, also indicate that a significant fraction of historical compounds might not be suitable for screening because of chemical degradation during long-term storage under nonideal conditions.[27,28] Indeed, confirmation of the chemical structure and composition of active samples is one of the standard early tasks involved in assessing hits from a screening campaign.

Compounds produced by early combinatorial chemistry methods were designed based on synthetic accessibility and diversity of structure and tended to be high molecular weight, lipophilic structures of questionable purity. The current emphasis on the quality of compounds produced by high-throughput synthesis, rather than on the sheer numbers, has led to libraries where drug-like properties are considered before synthesis is initiated and where design is often focused on providing leads against particular targets or target classes.

Compounds acquired commercially to augment screening collections are selected for diversity of structure and drug- or lead-like properties. However, since they originate in the public domain, these same compounds, or close analogs, are often present in many different screening collections and, if identified as lead structures, do not carry a satisfactory intellectual property position without substantial structural modification.[29]

Natural products tend to be structurally diverse and complex and, from a synthesis perspective, are often difficult to modify at sites that are relevant for SAR studies. As is apparent from Table 7.1, none of the screening-derived drugs that were approved over the past decade came from leads provided by the natural product world. A possible rationale is provided by Hann *et al.* who postulate an inverse relationship between molecular complexity beyond a certain level and the likelihood of encountering a productive binding event with a biological target. In other words, very complex molecules are less likely to forge a sufficient number of productive interactions with a target to overcome the many likely negative or unproductive ones.[20]

B. Assays

In the early 1990s, HTS was largely a manual process[30] with a throughput on the order of hundreds of samples

per week. Screening capacity has grown to the extent that collections of a million compounds or more can now be assessed in a month or less. The increased capacity has been facilitated by automation, high density plates and the movement of detection technology to fluorescence-based techniques. Statistical methods have been developed to assess the quality of any particular screen; the parameter *Z* which gauges signal reproducibility across the dynamic range of the assay is frequently used to quantify the robustness of an assay.[31] With 384 and 1,536 well plates now in routine use, miniaturization to low volume (uL) assays means that only minute amounts of compound are required for an individual test, and low milligram sample quantities can last for hundreds of screening campaigns.

Structural elements that often result in false and misleading positive activity are now well recognized; such functional groups include those that are chemically reactive and can act as alkylating, acylating or reducing agents. A composite listing of such groups is shown in Table 7.2, along with some additional structural features that are generally considered to be undesirable in screening samples.[28,32–34] These features may confer chelating, detergent-like or aggregation properties on a test sample[35,36] and can result in false positive activity through nonselective mechanisms. In all, a substantial experience base has emerged that now enables robust assays and the early identification of spurious activity based on artifacts.[37,38]

C. Hit-to-lead process

It would not be unusual for a screening campaign involving one million samples to generate several thousand samples that display activity above a meaningful threshold. Confirmation of both the activity and the identity of the active structures provides a set of confirmed screening hits. Whereas in the past identification of structures with acceptable molecular potency, selectivity, and patentability might have been sufficient to initiate LO,[22] it is now customary to provide a more detailed assessment of the liabilities and opportunities associated with any intended lead series via a process frequently referred to as "hit to lead." An important part of this process is the demonstration that an appropriate pharmacokinetic (PK) or pharmacological profile can be achieved in addition to satisfactory molecular potency.

Obstacles to a targeted PK profile, for example, poor permeability or rapid metabolism, can be identified through surrogates such as permeability in a Caco-2 assay or by *in vitro* metabolism as measured in microsomal or hepatocyte preparations. It is also increasingly common to provide *in vivo* PK data on representative structures.[39] Off-target liabilities such as hERG or CYP inhibition[40,41] should also be identified at this early stage – it is not necessary to fix all the issues that are identified, but data should indicate that there is a path forward during LO (Box 7.2).

TABLE 7.2 Functional Groups and Structural Features Undesirable for Molecules in a Screening Collection*

Reactivity	Structural
RO—OR, RS—SR, RN—NR,	Any element other than H, C, N, O, S, F, Cl, Br, I
RN—OR, RN—SR, RS—OR	More than six F, more than three Cl, Br, I
Anhydride, acyl halide,	Epoxide, aziridine or thiirane
Activated ester or thioester	More than one nitro group
Alkyl chloride, bromide, iodide	Any ring larger than eight-membered
Sulfonyl halide, sulfonate ester	Crown ethers
N=C=N, N=C=S, N=C=O, isonitrile	Linear polycyclic aromatic systems
Nitroso, diazo, thiocyanate	≥linear $(CH_2)_6$
Aldehyde, cyanohydrin, imine, chloramidine	>2 Ar—NH_2 groups (Ar = phenyl or naphthyl)
Michael acceptors	>2 Ar—OH groups (Ar = phenyl or naphthyl)
Alkyl—SH	Diacetylene, polyene
1,2- or 1,4-quinone	Trihydroxyphenyl
N-Halogen or S-Halogen	≥4 Acidic groups
Activated 2-haloheterocycles	≥4 Basic Nitrogen atoms
β-Lactam	>2 Quaternary amine
1,2-dicarbonyl	
S, O, Cl, or I atom carrying a positive charge	
Phosphoramide, phosphorane	

*Source: Compiled from Refs 28, 32–37

IV. EXAMPLES OF DRUGS DERIVED FROM SCREENING LEADS

By its nature, a screening approach to lead identification has the potential to identify not only structurally novel lead compounds, but also unexpected modes of action or allosteric inhibition.[42] In cases for which there is no prior experience with drugging a particular biological target or where the natural ligand is not amenable to rational medicinal chemistry processes, screening may provide the most effective method for lead identification. However, because of the

BOX 7.2 Progression from Screen to Lead

		Number	
Hit identification	**Compounds screened**	10^6	
	Confirmed active samples	10^3–10^4	
	Counter screens Dose-responsive Druglike or leadlike properties, no toxicophores Confirmed structure and purity		
	Selective confirmed hits	10^1–10^3	
Hit to lead	Demonstrated, exploitable SAR Tractable synthesis Solubility, ADME, toxicity profiling Demonstrated efficacy in cellular and/or pharmacological models Expanded selectivity profiling Clear patent strategy		
Lead optimization	**Lead series**	3–5	~1 year

similarity of screening collections and the attractiveness of particular targets and target classes, the potential to discover structurally and mechanistically novel leads is coupled with the possible identification of similar lead structures by competing research groups. The examples selected below highlight some of the opportunities and challenges offered by leads obtained from screening processes.

A. Reverse transcriptase inhibitors, nevirapine, efavirenz, and delavirdine

Through the 1990s a handful of drugs derived from leads identified by screening or HTS reached the market. Three of these, efavirenz (**2**), delavirdine (**6**) and nevirapine (**9**) (Figure 7.1) are AIDS therapeutics and target the viral reverse transcriptase enzyme (HIV-1 RT) via a novel allosteric mechanism of inhibition. The lead structures, all derived from historical corporate collections, are also shown in Figure 7.1 along with a summary of the major issues, in addition to improving potency, that were addressed during the LO campaigns.

A number patents and publications describe sedative or antidepressant properties for quinazolinethiones such as **3** related to the efavirenz lead **1**.[43] Chemical instability of the lead structure **1**, due to the masked ketone at the 4-position, was addressed by replacing the ethoxy group with a carbon linked substituent.[44] A focus on replacing the thiourea functionality because of potential toxicity led to urea analogs, and subsequent efforts were directed toward solving the low metabolic stability of the *N*-methyl group. This was attained by a switch to the benzoxazinone system present in efavirenz, in which –O— is replaced –*N*(Me)–; however, this scaffold change was only enabled after considerable SAR studies had identified 4-position substituents that retained potent enzyme inhibition across the scaffold switch.

The lead **4** for delavirdine (**6**) was discovered in a screened set of 1,500 computationally diverse representatives of the Upjohn compound collection. There is only one literature ref.[45] to the delavirdine lead structural type, exemplified by compound **7**, prior to the disclosure of RT inhibitory activity for this class. Rapid SAR expansion of the lead was enabled by *N*-benzyl connectivity and many alkylated and acylated variations of the upper portion of the piperazine scaffold were explored. Ultimately the acylindole, initially bearing a 5-methoxy substituent as in the first clinical candidate atevirdine (**5**), emerged as preferred. This was found to be metabolically labile and was subsequently replaced with the methylsulfonamide group. Early work also identified the *N*-ethyl substituent of the lead as a potential metabolic liability and, although this pattern was retained in the first clinical candidate, it was replaced by the *N*-isopropyl substituent in the approved drug, delavirdine (**6**).

Structures related to the nevirapine lead **8** are well represented in the patent and scientific literature, since the core system is similar to that in the approved drug pirenzepine (**10**). Initial SAR efforts were driven largely by metabolic instability associated with each of the *N*-alkyl substituents. An acceptable profile was achieved with two changes. First, by modifying the attachment point of the methyl group from the 5- to the 4-position the extent of

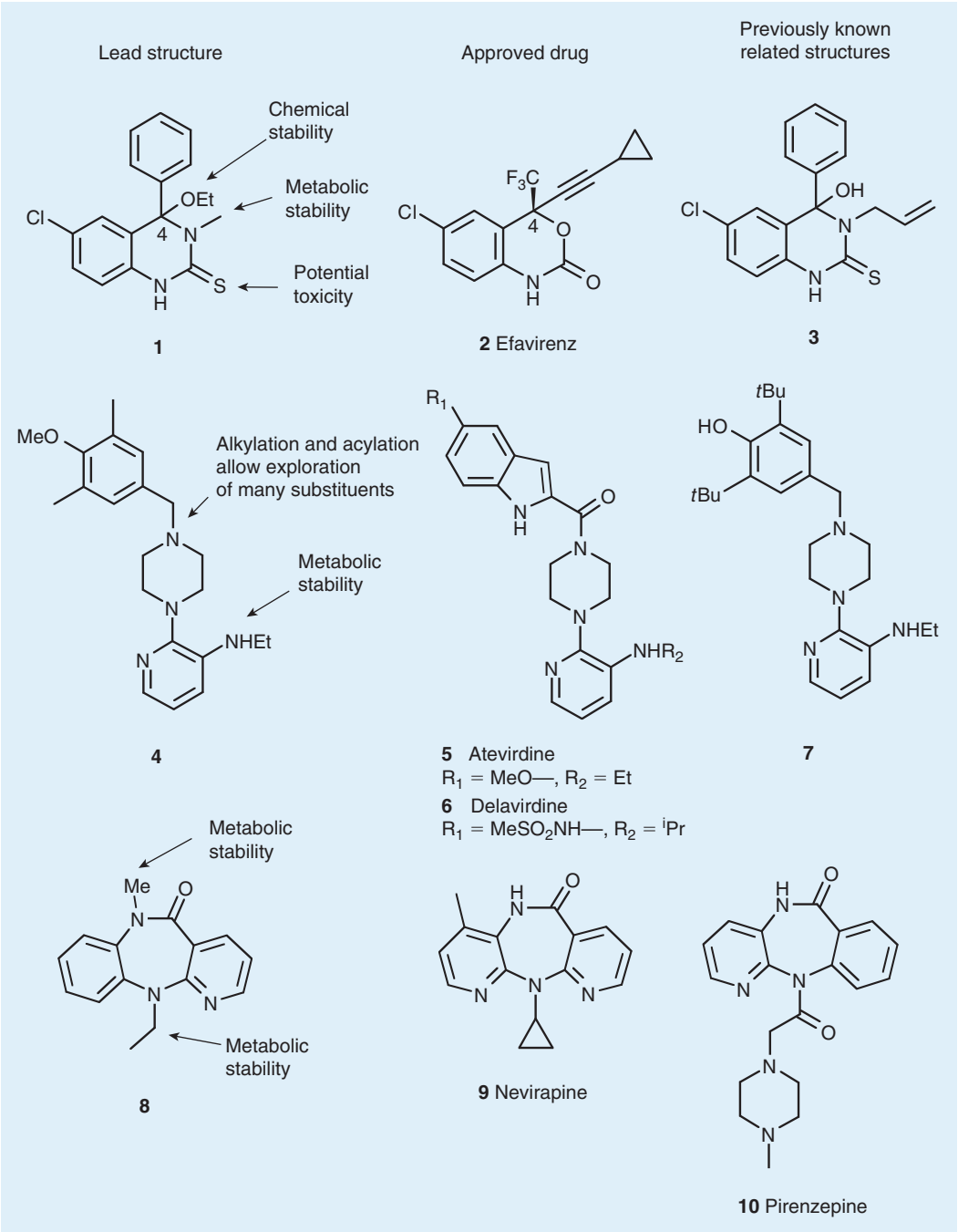


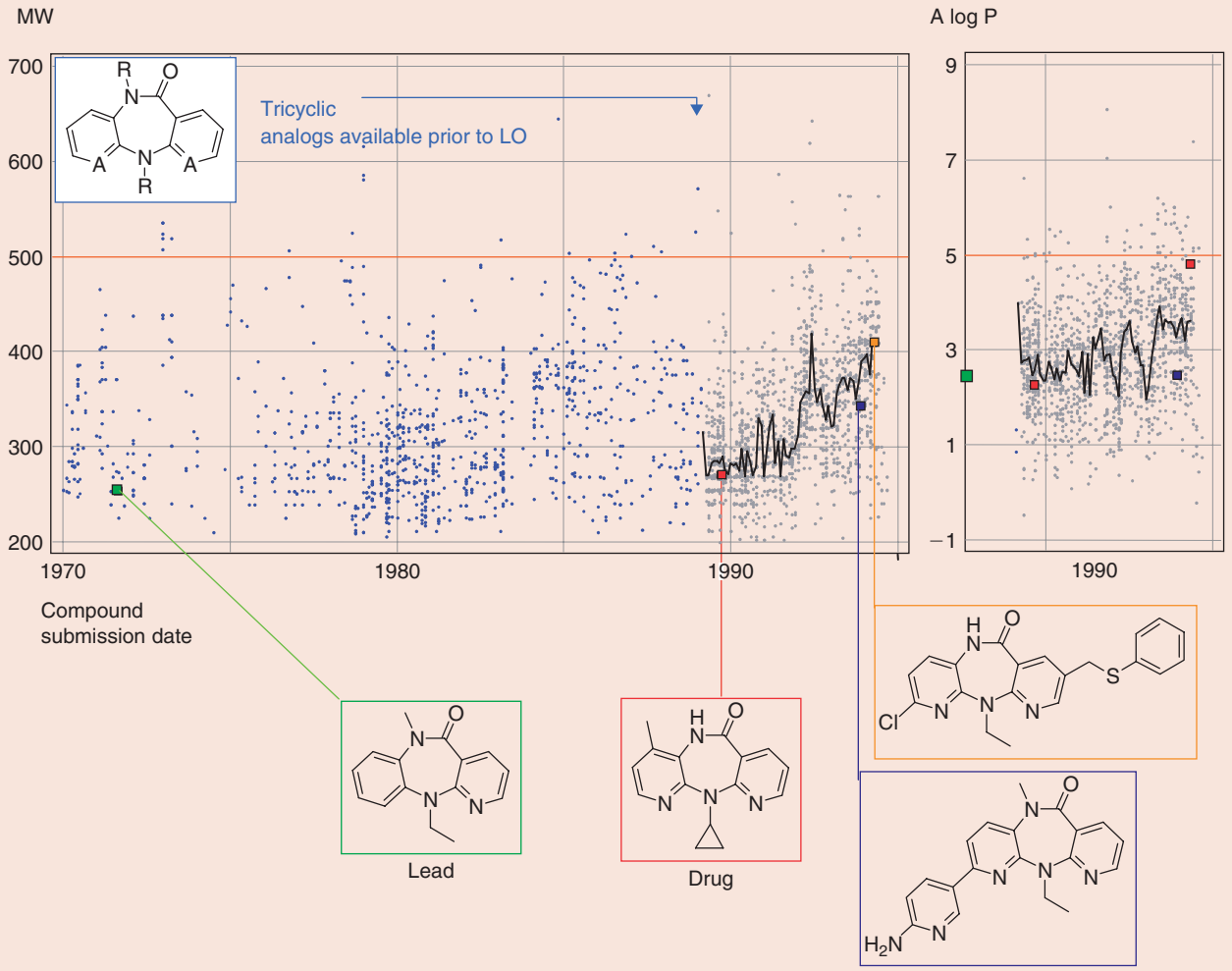
FIGURE 7.1 HIV-1 reverse transcriptase inhibitors.

metabolism was significantly decreased and this change also led to an improvement in potency. Replacement of the *N*-11 ethyl substituent with a cyclopropyl group also provided an improved profile. Additionally, the introduction of a second nitrogen atom in the tricyclic ring system gave a further boost in potency and also improved solubility and provided the drug candidate nevirapine, **9**.[46]

It is important to emphasize that, for these three examples, the lead structures, which act by a unique allosteric mode of inhibition, would not have been discovered by any other method available at the time, that is, were it not for the application of a screening approach to lead identification this class of drugs might not have emerged to find use in the clinic (Box 7.3).

BOX 7.3 An Alternative Representation or the Evolution of the Nevirapine Series

This illustration of property change over time highlights the importance of corporate compound collection for the initial rapid progression of the SAR studies. There was a wealth of potential structure/activity information available even before synthesis was initiated. The attractive lead-like properties of the initial structure allowed substantial latitude for increases in MW and lipophilicity during LO. After the identification of nevirapine, subsequent medicinal chemistry efforts revolved around generating activity against multiple resistant mutant RT enzymes.[47,48] Improved potency profiles were achieved through additional substituents on the dipyridodiazepane ring system, although these usually came at the cost of metabolic or other liabilities.



B. Endothelin antagonists, bosentan, sitaxentan, edonentan, and ambrisentan

The examples above illustrate a situation where screening against a target gives multiple structurally distinct lead classes. Alternatively, screening may give identical or very similar lead structures to different organizations. This is not an unlikely scenario, given that a substantial portion of any screening collection may be composed of commercially acquired compounds. In the examples shown in Figure 7.2 from endothelin antagonist programs, screening based approaches identified the lead structures that resulted in sitaxentan, bosentan, ambrisentan (all approved drugs) and edonentan (a clinical candidate). Two of these discovery campaigns began from very similar lead structures.

A directed screening approach, in which computational searching based on the pharmacophoric elements apparent in the natural ligand was used to direct the purchase or selection of compounds for screening, identified sulfisoxazole (**11**) as a moderately effective inhibitor of binding of

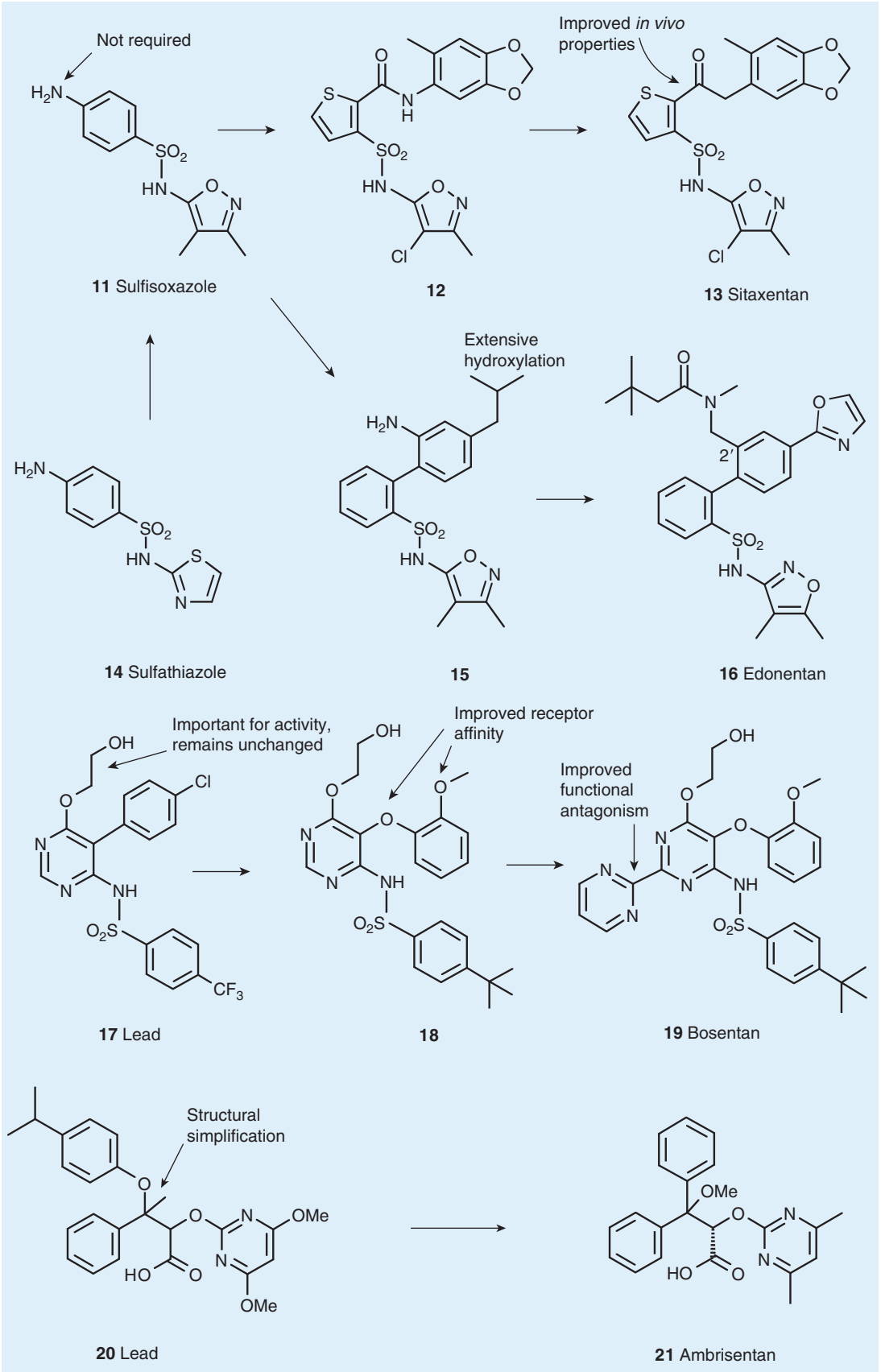


FIGURE 7.2 Endothelin antagonists.

radiolabeled endothelin 1 to the ET_A receptor.[49] SAR studies showed that the aniline moiety was not required for activity and the phenyl ring was replaced with the isosteric thiophene.[50] Subsequent elaboration on this ring gave molecules, such as the amide **12**, which demonstrated inhibition at the low nM level.[51] These compounds, however, demonstrated poor bioavailability due to cleavage of the amide bond and a search for a stable metabolically stable replacement eventually identified the ketone linker present in sitaxentan (**13**).[52]

The same lead structure, sulfisoxazole (**11**), was also identified by Bristol Meyers Squibb, via the screening hit sulfathiazole (**14**).[53] In this instance modification of the aniline led, via a naphthalene ring, to the substituted biphenyl **15**;[54] however, preclinical PK studies indicated that the attached isobutyl group was subject to extensive hydroxylation.[55] Replacement of this group with an isoxazole ring imparted metabolic stability, and expansion of the SAR at the tolerant 2′-position provided the clinical candidate edonentan (**16**).[56,57]

Two additional approved endothelin antagonists which derived from HTS leads have progressed to the market – bosentan (**19**) and ambrisentan (**21**). The lead **18** for bosentan,[58] although not a sulfa drug, bears a structural resemblance to sulfisoxazole and sulfathiazole, although in more elaborated form. Substances closely related to **18**, differing only in the chloro and trifluoromethyl substituents, are exemplified in the patent literature from Hoffman La Roche as blood sugar lowering or antidiabetic agents devoid of antibacterial activity [59] and it is possible that the lead was originally synthesized based on a sulfa drug precursor structure. It is noteworthy that the lead remained part of the screening collection for more than 20 years before it emerged as the starting point for bosentan. Since the lead selection criteria included demonstrated bioavailability and *in vivo* activity, the optimization process focused on improving potency which was attained by the modifications shown.[58]

In the case of ambrisentan (**21**), the lead structure **20** was originally synthesized as an herbicide and is similar to many such compounds patented in the late 1980s. In addition to improving potency, one early goal was to simplify the lead structure by incorporating identical substituents at one of the two chiral centers. The strategy proved remarkably successful in that a straightforward diphenyl substitution at the left-hand side chiral center provided both simplification and improved potency. Ambrisentan is a rare example of an optimized drug that is smaller and less complex than the original screening lead.[60]

C. Raf kinase inhibitor, sorafenib

One notable example of HTS providing a lead structure with a novel chemotype and a novel mechanism of action is presented in the discovery of sorafenib (**28**), Figure 7.3, which was recently approved as an anticancer agent. An HTS conducted against Raf-1, a kinase implicated in cancer cell proliferation, provided the thienylurea **22**, a commercially available compound, as a lead structure (Figure 7.3).[61] Among many early structural changes, it was found that methyl substitution at the 4-position of the right-hand phenyl ring, as in **23** provided enhanced potency, although it proved difficult to make further progress with the thiophene left-hand ring in place. Since the structural class was amenable to combinatorial chemistry methods, many combinations of left- and right-hand side variations were made and tested. A significant discovery emerging from this approach was that the thiophene could be replaced with an isoxazole ring system as in **26**. The particular combination of left- and right-hand side fragments in **26** deviated from predicted activity in that the corresponding analogs with the single point changes, **24** and **25**, showed little activity against the target. This is an instructive example of the nonlinear nature of SAR progression and provides a clear example of the power of combinatorial chemistry applied during LO. In this instance, it provided the opportunity to discover an unexpected synergism between pharmacophoric elements. Introduction of a pyridine ring at the right-hand side improved solubility, and incorporation of a disubstituted phenyl ring on the left side provided additional potency as in **27**. Finally, introduction of the carboxamide substituent on the pyridine ring as shown for sorafenib (**28**) provided a further boost in potency thought to be due to a favorable hydrogen bonding interaction with the target enzyme.

At the time of discovery, the thienylurea lead structure **22** represented a novel structural class for kinase inhibition. Concurrently, similar structures were identified as inhibitors of P38 MAP kinase,[62,63] an enzyme involved in the regulation of inflammation. Subsequently, it was shown that sorafenib inhibits Raf-1 by binding to an inactive conformation of the enzyme, a mechanism of action that has also been observed for other kinase inhibitors.[64,65]

V. PRACTICAL APPLICATION, RECENT EXAMPLE

A. IKK inhibitors

The examples above represent successful exploitation of leads generated from various screening processes. Of necessity, since many of the drugs described have reached the market, they represent discovery processes that were in use a decade or more ago. An instructive example of more recently applied processes is illustrated in Figure 7.4. Screens against IKKβ, a kinase involved in the regulation of the gene transcription factor NF-κB, produced very similar, if not

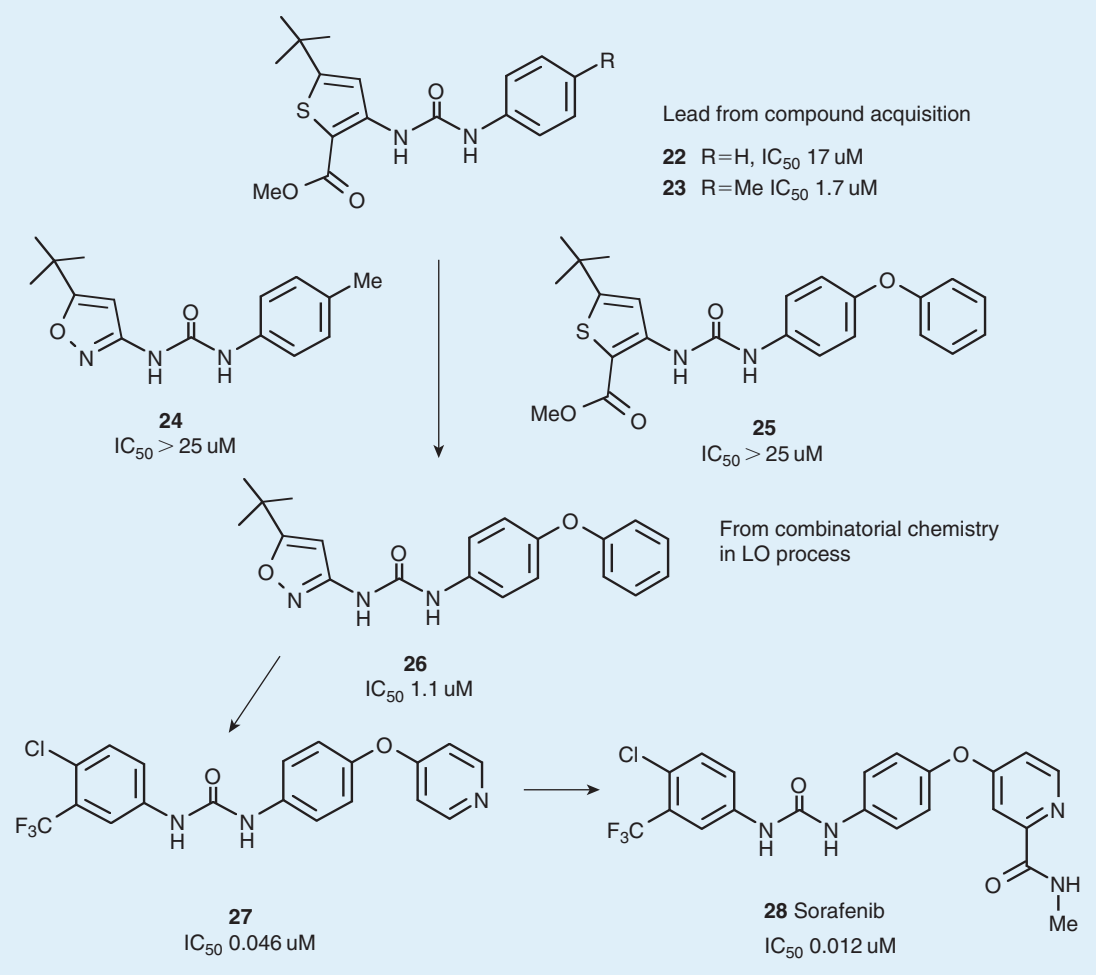


FIGURE 7.3 Raf-1 kinase inhibitors.

identical lead structures for at least four different organizations, Boehringer Ingelheim,[66] AstraZeneca,[41,67] SmithKline Beecham[68] and Pharmacia[69] and two publications have appeared detailing the processes used to evolve the screening hit into lead series.

At AstraZeneca, the aminothiophenecarboxamide screening hit **28**, a commercially available compound, and the urea analog **29** were confirmed as a viable hit structures. Liabilities identified in progressing toward lead status were poor solubility and poor metabolic stability. Combining structural features of the two hits gave molecules with substantially improved molecular potency as in **30**. Incorporation of central ring heterocycles predicted to improve solubility, as in compounds **31** and **32**, instead gave substantially decreased potency. Although the solubility profile could not be significantly improved, representative final molecules showed acceptable *in vivo* exposure, and poor solubility was ultimately not an impediment to achieving LO status. Substitution on the amide nitrogen abolished potency, SAR information that was consistent with a binding model in which this group engages a key hinge-binding interaction with the target enzyme. Additional SAR studies focused on improving metabolic stability and identified electron withdrawing substitution on the phenyl ring that conferred an acceptable profile, exemplified by the *p*-fluorophenyl derivative **33**. Overall this molecule fulfilled all lead criteria aside from acceptable solubility, but since the solubility profile did not adversely affect oral bioavailability the series progressed to LO.

Screening at Boehringer Ingelheim identified the same hit **28** along with the fluorophenyl analog **34**. Criteria applied to the selection of these compounds as validated hits included selectivity, drug-likeness, tractability of syntheses and support for interaction with the target. Bicyclic analogs (**35**) and (**36**) clarified the important hydrogen bonding interactions with the hinge region of the kinase and, along with additional analogs, provided sufficient information to construct a useful pharmacophore model.[66] Although monocyclic scaffolds analogous to those examined by AstraZeneca were also made and tested, the hit-to-lead effort ultimately focused on bicyclic scaffolds exemplified by **37** as offering the best opportunities for exploring

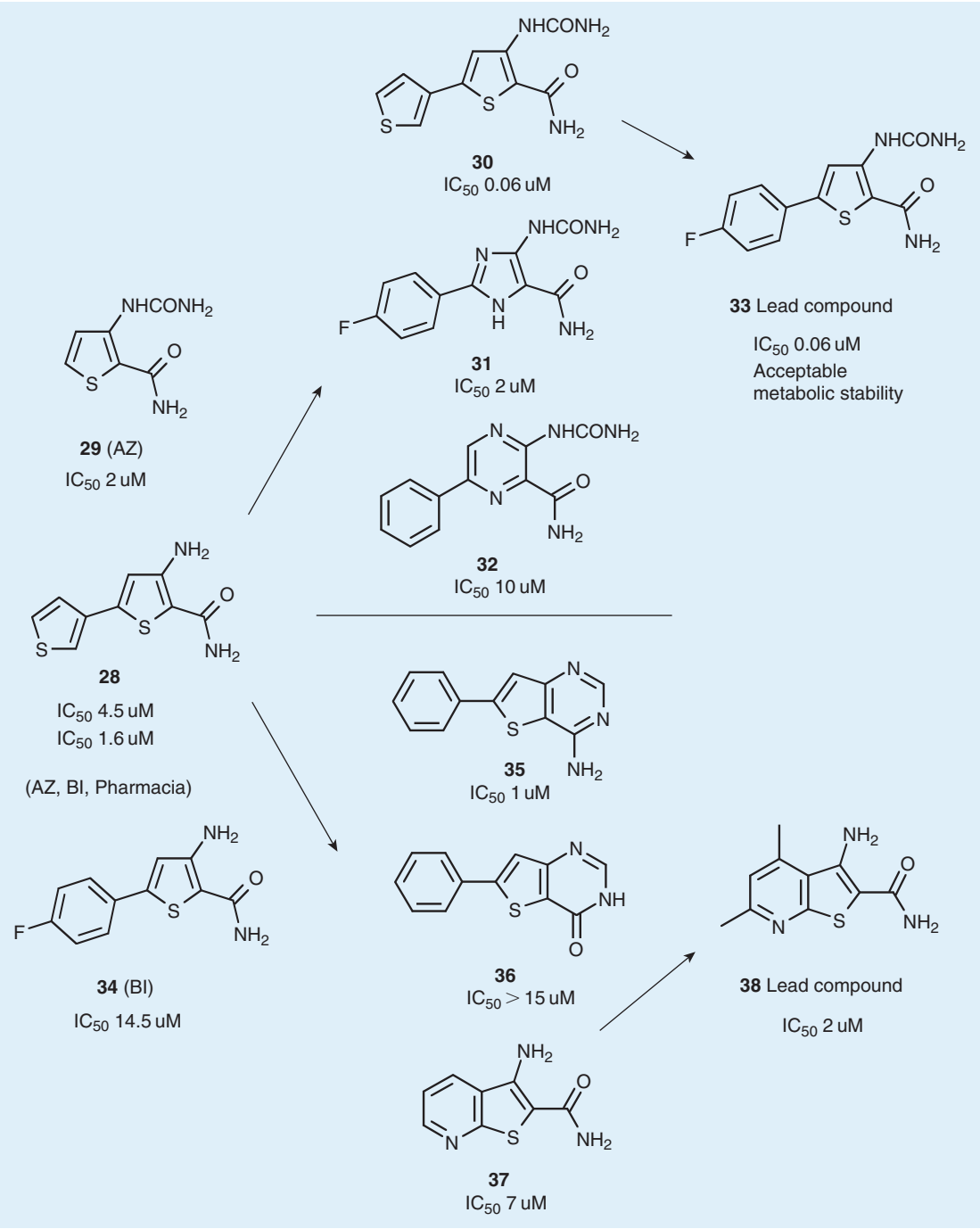


FIGURE 7.4 IKK inhibitors.

the potential interactions identified by the pharmacophore model. Subsequent improvement in potency was achieved by substitution on this thienopyridine scaffold. Overall, this scaffold offered substantially improved metabolic stability and CYP-450 inhibition profiles over the original hit thiophene scaffold, along with a clearer IP position, and progressed to LO.

VI. CONCLUSION

Notwithstanding its relatively recent introduction, it was anticipated that HTS, in conjunction with combinatorial chemistry, would provide a large positive impact on drug discovery productivity and perhaps even identify potential drug candidates directly from large libraries of compounds.

TABLE 7.3 The Impact of High Throughput Screening

*Patent**	1985–1990	1990–1995	1995–2001
Drugs	Nevirapine	Tirofiban	Gefitinib
	Delavirdine	Bosentan	Cinacalcet
	Mozavaptan	Efavirenz	Sorafenib
	Sivelestat	Conivaptan	Dasatinib
		Tipranavir	Sitagliptin
		Sitaxentan	Sunitinib
		Aprepitant	Ambrisentan
			Maraviroc
Targets	HIV-RT, elastase	HIV-protease, ET_A, NK-1,	DPP-IV, Kinases,
	Vasopressin V2	Fibrinogen receptor	Ca^{2+} receptor, CCR5
First in class	1	3	6
Technologies	Membrane preparations	Fluorescence	Cell-based fluorescence
	Radioisotopic detection		
	Colorimetric detection		
Numbers	25,000 @ 200 week	100,000 as mixtures	200,000
Lead sources	Corporate historical	Drug	Compound acquisition

*Priority date.

However, through the 1990s only a small number of approved drugs originated in screening processes. For example, most drugs approved in 2000 derived from analog-based discovery approaches,[70] a situation that was not significantly different from that described earlier by Freter.[5]

Table 7.3 provides an alternative perspective on the list of HTS-derived drugs presented in Table 7.1. The timeline here is focused on drug discovery rather than patent approval date and additional target and technology information is included. It is clear that the many of the targets that have been successfully addressed by lead identification through screening approaches have provided first-in-class drug candidates. This compilation does not reflect the capability of current screening technologies and does not incorporate the impact of such initiatives as the recently implemented "New Pathways to Discovery" component of the National Institutes of Health (NIH) roadmap. This initiative which will expand access to HTS capacity beyond pharmaceutical companies to basic research institutions and is expected to impact not only the availability of tool compounds but also the delivery of therapies for rare diseases which are often not attractive to the private sector. Additionally, while further increases in screening capacity against individual targets can be expected, high-content screening which quantifies cellular and subcellular events through fluorescence microscopy and image analysis is also progressing into the high throughput world and promises to further expand the quantity and quality of information that will be available to assist lead identification.[71]

In the past, natural products, receptor agonizts, enzyme substrates, and literature compounds were the main sources of starting points for drug discovery campaigns. However for many biological targets the relevant enzyme substrates or receptor ligands are not attractive starting points for medicinal chemistry and, in these cases, HTS is proving to be a key resource for the generation of novel tractable lead series.[72]

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