Available online at www.sciencedirect.com
ScienceDirect
Current Opinion in Chemical Biology

Fragment-based activity space: smaller is better

Thomas Hesterkamp[1] and Mark Whittaker[2]

Fragment-based drug discovery has the potential to supersede traditional high throughput screening based drug discovery for molecular targets amenable to structure determination. This is because the chemical diversity coverage is better accomplished by a fragment collection of reasonable size than by larger HTS collections. Furthermore, fragments have the potential to be efficient target binders with higher probability than more elaborated drug-like compounds. The selection of the fragment screening technique is driven by sensitivity and throughput considerations, and we advocate in the present article the use of high concentration bioassays in conjunction with NMR-based hit confirmation. Subsequent ligand X-ray structure determination of the fragment ligand in complex with the target protein by co-crystallisation or crystal soaking can focus on confirmed binders.

Addresses
[1] Evotec AG, Schnackenburgallee 114, D-22525 Hamburg, Germany
[2] Evotec (UK) Ltd., 114 Milton Park, Abingdon, Oxfordshire OX14 4SA, UK

Corresponding authors: Hesterkamp, Thomas (thomas.hesterkamp@evotec.com) and Whittaker, Mark (mark.whittaker@evotec.com)

Current Opinion in Chemical Biology 2008, **12**:260–268

This review comes from a themed issue on
Molecular diversity
Edited by Gerry Maggiora and Christopher Hulme

Available online 19th March 2008

1367-5931/$ – see front matter

DOI 10.1016/j.cbpa.2008.02.005

Introduction

Fragments represent smaller, less complex, molecules than either drug compounds or typical lead series compounds. It is now widely acknowledged within the pharmaceutical and biotech industries that weakly active fragment hit molecules can be efficiently optimised into lead compound series if structural insight is obtained at the outset for the binding interaction between each fragment hit and the target protein of interest. This is supported by recent reports of the progression into human clinical trials of drug molecules developed from weakly active fragment starting points [1•].

There have been a number of excellent reviews published in the past year that cover fragment-based drug discovery in general [2••,3••], particular approaches to fragment screening [1•,4,5•], as well as the specifics of implementation within different organisations [6•,7••,8].

Here we focus on chemical diversity in relation to methods for screening fragments and highlight how this may impact on fragment-based drug discovery.

Chemical diversity and fragments

Fragment-based drug discovery can explore drug-like chemical diversity space in an efficient and effective manner. This is demonstrated both by finding novel starting points for targets for which it has previously been found to be difficult to find small molecule ligands [9•] and by the speed with which optimisation can be accomplished. The two key factors are, firstly, the relatively good coverage of fragment chemical diversity space during the screening stage and, secondly, that drug chemical diversity space is explored in an efficient iterative fashion during the optimisation stage as fewer combinations need to be evaluated than through a purely random screening and undirected optimisation approach [6•]. For example, a fragment collection of 10 000 molecules may virtually represent the diversity of one billion molecules if one considers the combinatorial power of fragment merging or linking (e.g. by assuming two adjacent binding sites to which fragments bind and 10 different possibilities of fragment linking) [10], but only a small part of the larger chemical space defined by fragment merging and linking needs to be explored in the structure-directed elaboration of fragments into leads.

A key question is 'When is a molecule a Fragment?' [3••]. The most widely accepted definition for fragment molecules is provided by the rule of 3 according to which fragments are compounds with molecular weight $\leq$300 Da, clogP $\leq$3 and three or less hydrogen bond donors [11]. This is a derivation of the Lipinski rule of 5 for oral bioavailability of molecules [12] and is based on the experience of the Astex group in finding fragment hits through screening by high throughput crystallography. However, various research groups have applied variations of the rule of 3 criteria as well as applying additional criteria [13]. Another key question is 'How do you select the best fragment for optimisation?'. A very useful concept that can be applied to help with selecting the best fragments for optimisation is 'ligand efficiency', which relates target affinity or potency to molecular size [14,15]. Ligand efficiency (LE) has been developed from the concept of Kuntz *et al.* [16] on the maximal affinity of ligands and represents the binding energy per heavy atom in a molecule. This is equal to the free energy of binding of a ligand to a target protein divided by the non-hydrogen atom count (NHC) of the ligand (i.e. $LE = -\Delta G/NHC \approx -RT\ln(IC_{50})/NHC$) [14]. Importantly the binding energy contribution per non-hydrogen atom tends to

level off when reaching a NHC of 15 or more, which equates to a fragment-like molecule of ~250 Da. Thus, rather than focusing on the potency of a hit molecule, fragment-based drug discovery gives access to low molecular weight *efficient binders* where the optimisation process can concentrate on improvements in potency and other desirable attributes without an immediate concern about increases in molecular weight. Not all fragment hits will display high ligand efficiency in their interaction with a particular target protein and so the concept of ligand efficiency is particularly useful in selecting which fragments to take forward into optimisation. It has been suggested that fragments selected for optimisation should exhibit a LE in excess of 0.3 kcal mol^{-1} per non-hydrogen atom since this corresponds to the LE of a fully optimised drug of molecular weight 500 (typical number of non-hydrogen atoms $\approx$ 38) and IC_{50} of 10 nM, and it is assumed that optimisation to drug molecules will proceed in a linear fashion by the addition on average of 0.29 kcal mol^{-1} binding affinity per additional non-hydrogen atom [14,17••,18].

How one chooses to define fragments has an impact on chemical diversity [19]. It has often been argued by the proponents of fragment-based drug discovery that a relatively small number of low molecular weight fragment molecules can provide a higher degree of sampling of the chemical diversity space for fragments than a very large number of higher molecular weight compounds is able to sample the respective chemical diversity space for drug-like compounds [20•]. In support of this theoretical studies by Reymond and co-workers suggest that the number of possible organic compounds increases exponentially with the square of the number of atoms [21,22], and it has been further estimated by Guida and co-workers that the universe of organic compounds containing up to 30 C, N, O and S atoms is in excess of 10^{60} different molecules [17••,23]. Furthermore, Hann and co-workers have argued that lower molecular weight molecules exhibit reduced complexity than the molecules in drug-like collections and have developed a model to rationalise ligand–receptor interactions in the molecular recognition process [24•]. According to this analysis it was shown that the theoretical probability of a useful interaction falls dramatically with increasing molecular complexity of the ligand. It can be argued that drug-like collections such as typical HTS screening decks contain molecules that are too complex to yield useful binding events in a reasonable relationship to the size of the compound file. These arguments are well reasoned but the decisions made in the implementation of fragment-based drug discovery will affect the extent to which the chemical diversity space for fragments is effectively covered. This is due to some general limitations inherent in the fragment-based approach to drug discovery and also restrictions imposed by the different techniques used for fragment screening. The general limitation that applies to all fragment screening methods is that each fragment molecule needs to have a relatively high aqueous solubility in order to be screened at the high concentration required to determine the expected weak binding interactions. This will result in the chemical space of more lipophilic fragment molecules not being screened. While this aspect is encoded to some extent in the rule of 3 through the limitation of lipophilicity to those compounds with clogP of 3 or less, we and others have applied *in silico* QSAR models for aqueous solubility to restrict fragment selection to those compounds whose solubility is above a certain threshold. In selecting our fragment library for screening by high concentration bioassay we have only selected those compounds predicted to have an aqueous solubility in excess of 1 mM [25] while Hubbard and co-workers at Vernalis apply a limitation of 2 mM [26,27•]. The Hubbard group have reported that the application of such an *in silico* filter for aqueous solubility can remove in excess of 50% of commercially available fragment molecules from consideration [26]. However, this constraint to fragment chemical diversity space does have the advantage that fragment optimisation starts from compounds with good aqueous solubility that gives scope for the introduction of lipophilic moieties, as is typical during the course of medicinal chemistry optimisation, while still maintaining drug-like properties within acceptable limits [28,29].

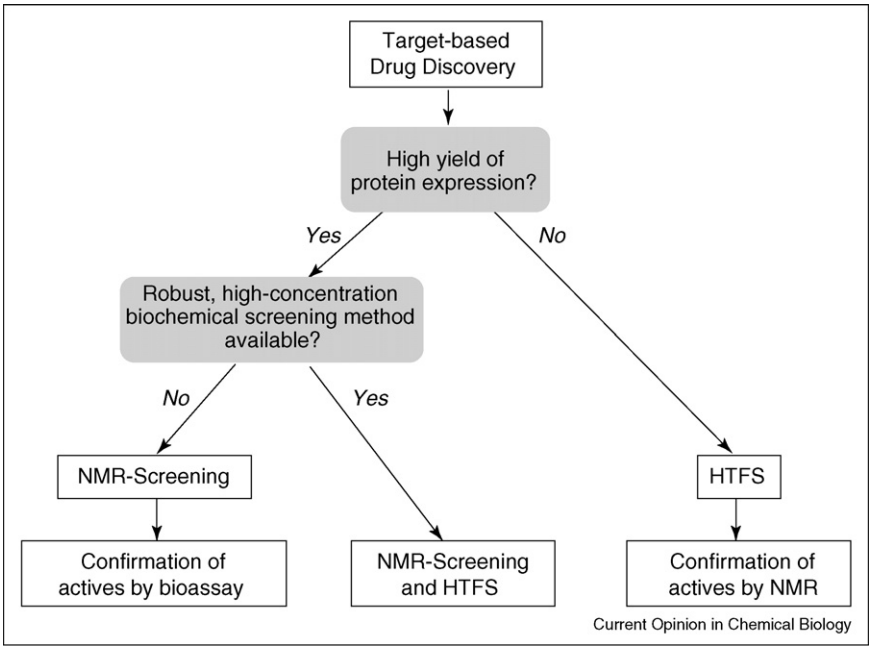
Impact of fragment screening method on diversity

While the theoretical probability of target interaction increases with lowering the ligand complexity, Hann and co-workers considered that measuring a weak interaction would be more difficult. Hence, the authors introduced the term 'Probability of useful event', which is a bell-shaped curve (too small ligands are missed in screening and too big ligands have a poor probability of target interaction). On the basis of this premise, the selection of the fragment screening technique is of key importance. A plethora of methods exists for fragment screening, and these have been comprehensively reviewed elsewhere [1•,4,5•,17••,25]. The question that we wish to focus on here is how each method predetermines the extent of exploration of chemical diversity space for fragments. While there are some very specific limitations imposed by various fragment screening methods there are two general factors that have an impact for all methods. The first is sensitivity of the screening method and the second is throughput. Sensitive screening methods enable weakly active fragment molecules of lower molecular weights to be identified as hit compounds and so fragment libraries with a lower molecular weight range can be used. On the contrary the use of a low throughput screening method necessitates the use of a smaller fragment library with a concomitant sparser coverage of fragment chemical diversity space.

Perhaps the most elegant method of fragment screening is by X-ray crystallography in that it provides directly structural information on the interaction between fragment ligands and the protein target [30]. However, owing to the method's low throughput, even when fully automated, the technique can only be effectively applied to targets for which a robust crystallographic system is available that allows soaking of preformed crystals with fragment cocktail mixtures of up to 10 compounds at high concentrations [31]. This requirement imposes two key limitations. The first limitation is that the number of fragment compounds that can be evaluated per screening campaign is typically limited to no more than 1000. This is in spite of the efforts that have been put into fragment cocktail design to obviate the need for tedious deconvolution experiments to identify which compound is giving rise to the observed ligand electron density in the X-ray crystallographic studies. An approach to facilitating direct identification of the binding fragment from a cocktail soaking experiment is to ensure that fragments are selected to ensure maximum shape diversity in each cocktail. The team at SGX Pharmaceuticals designed their fragment library for X-ray crystallographic screening so that approximately one-half of the compounds in the fragment library contain one or more bromine atoms (arylbromides). The presence of this halogen permits unambiguous bromine atom identification in experimental electron density maps, since the X-ray wavelength can be adjusted during the diffraction data collection to allow detection of anomalous dispersion signals unique to bromine. Furthermore, it is argued that the aryl bromides can be used as synthetic handles to facilitate synthetic elaboration of the screening hits. This is a very ingenious premise, but the lipophilicity of aryl bromides (the introduction of bromine onto an aryl ring increases cLogP by between half to one log unit) will lower aqueous solubility and tend to result in the bromine atom interacting with lipopophilic pockets within the protein target such that there is not a suitable vector for the direct elaboration of the binding fragment by exploiting the rich chemistry that is available for aryl bromides. Nevertheless, the SGX group has reported examples where this approach has been successful [20•]. In contrast to fragment screening by X-ray crystallography, screening by NMR or biochemical assay does not impose any additional requirements for the chemical matter to be screened. However, screening by NMR [4] remains a relatively low throughput method in comparison to screening by biochemical assay. In NMR screening, as when screening by X-ray crystallography, cocktail mixtures are used to improve throughput. However, in contrast to screening by X-ray crystallography larger numbers of compounds can be routinely screened; for example, the Abbott group screens up to 12 000 compounds by NMR methods in their fragment programmes [32]. This clearly allows a larger exploration of chemical diversity space than is possible by screening using X-ray crystallography alone. Other techniques such as using biochemical assays for high concentration screening of fragments have no limitations on throughput since high throughput screening devices are used [5•,7••,17••,25,33•] and so greater exploration of fragment chemical diversity is possible.

The second limitation to screening fragments by X-ray crystallography is that there is a significant possibility of missing active fragments owing to the protein being locked into a conformation, in the crystals used for the soaking studies, that does not allow the interaction of fragments that require induced fit to bind. Although, no data are available on the false negative rate for fragment screening by X-ray crystallography it may be significant for certain targets. Abbott scientists reported that while initial fragment hits could be effectively discovered by soaking preformed crystals of dihydroneopterin aldolase with fragment cocktails, the crystal structures of more active analogues, prepared from the initial fragment hits, could only be obtained by co-crystallisation studies [34]. This concern of missing active fragments has resulted in a general consensus approach to emerge 'whereby the fragment library is pre-screened with a relatively rapid and sensitive technique before being characterised structurally' [6•]. Such a triaging approach allows X-ray crystallographic studies to be performed by both soaking preformed crystals and by co-crystallisation studies. It should be noted that Astex, an early proponent of screening fragments by crystal soaking, now routinely uses NMR screening of fragments as a pre-filter to performing structural studies by X-ray crystallography [31] as does the Vernalis group [6•]. The approach that we have adopted at Evotec is to employ high concentration biochemical assays [25] in concert with NMR screening [35] to triage hit fragments before detailed structural determination [36]. Biochemical assays identify fragment hits with pharmacological relevance from the outset, that is, inhibitors of the target enzyme or competitors to biological binding partners. In addition, biochemical screening allows use of full-length proteins at low, typically nanomolar concentrations frequently in a near physiological environment with auxiliary proteins, substrates, bio-membranes and other co-factors associated. Hence, high concentration biochemical assays sample the biologically active more relevant form of enzymes and other protein/co-factor complexes. By contrast, NMR and other biophysical assays are routinely conducted on truncated protein domains at elevated micromolar target concentrations typically in the absence of substrates and other auxiliary macromolecules. Such clean biophysical assay systems enable robust screening at high micromolar to millimolar fragment concentration and the detection of particularly low molecular weight ligands because interference with auxiliary assay components does not cause false positives. Moreover, the binding site resolution of protein-detected NMR assays ensures that ligands binding only to the active or an allosteric site of interest are followed up, while non-specific

Figure 1



Decision tree for selecting the optimal combination of NMR and biochemical screening methods for fragments (HTFS is high throughput fragment screening by biochemical assay).

binders and compounds that give rise to protein precipitation are immediately eliminated. Consequently, protein-observed NMR screening identifies artefact-free binders that can be of low molecular weight. In fact protein-observed NMR has been positioned as a gold-standard technique to assess the 'drugability' of a target, meaning the likelihood to develop pharmaceutically relevant lead molecules for a target [32]. From a more pragmatic point of view researchers using biophysical fragment screening methods will use bioassays as an orthogonal method to check whether fragment hits exhibit biologically relevant activity and because bioassays will be required in every medicinal chemistry compound optimisation programme to drive the SAR. Vice versa bioassay fragment hits may be subject to ligand detected NMR experiments (or alternative biophysical techniques [5•]) as an orthogonal technique to confirm binding before proceeding into X-ray crystallography. We have developed a decision tree to help with the selection of the optimal fragment screening strategy (Figure 1).

Case studies

In a recent review by Alex and Flocco a summary is given of 68 fragment hits reported in the literature that have been progressed into lead compounds [3••]. Twenty-one of these fragment hit molecules were discovered by bioassay (HTS and biochemical screening), 15 by NMR screening and 14 by screening by X-ray crystallography. For those fragment hits that the reviewers were sufficiently confident in the reported activity we have calculated the average potency for fragment hits discovered by bioassay and by NMR screening to be approximately 2 mM each whereas the average potency for fragment hits discovered by X-ray crystallography we calculate to be 0.5 mM (Table 1). While it can be argued that with such low numbers of compounds the merit in such a comparison is not particularly useful, it is nonetheless surprising that there is so little difference between the techniques in terms of average potency of the hits that are selected for follow-up. We have also calculated average ligand efficiencies (LE) for this data set and find that the average for the fragment hits discovered by bioassay is 0.5, by NMR screening 0.3 and by X-ray crystallography screening 0.4. Considering that these LEs are derived from different types of results such as IC_{50}, K_i, and K_d we conclude an equivalency of the primary screening techniques in terms of hit identification sensitivity. We further conclude from this that the key distinctive feature between the three main methods for screening fragments is throughput. The higher throughput of bioassay methods enables more fragments to be screened and hence greater evaluation of fragment diversity space than for the other methods. We consider that using a relatively large diverse fragment library confers many advantages.

The targets that tend to be progressed in fragment-based drug discovery are those for which a robust crystallographic system can be readily developed. This can lead

Table 1

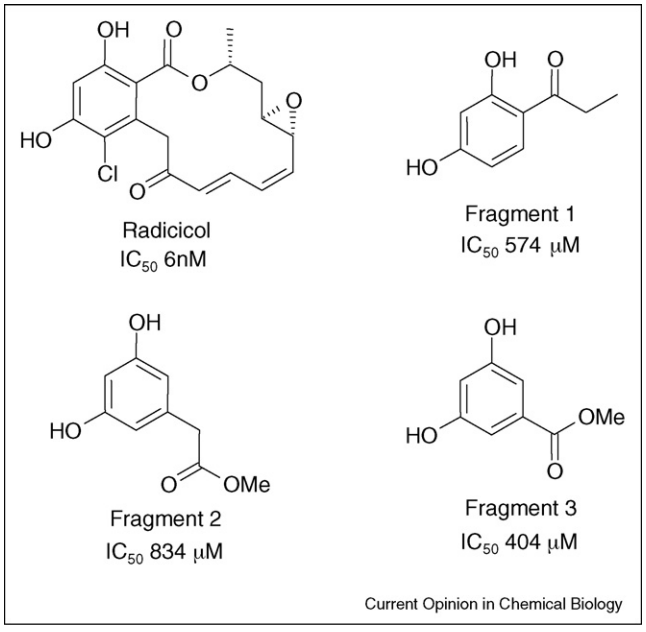
Comparison of the three main methods for fragment screening

Fragment screening method	Typical size of fragment library screened	Information provided	Activity ranges of hits (mean) (μM)[a]	LE ranges of hits (mean)[a]	Limitations with respect to diversity
X-ray crystallography	≤1 000 as mixtures of 4–10 compounds	3D-structural information on fragment target interaction	10–2 500 (450)	0.26–0.62 (0.41)	Small libraries only. Fragment hits may be missed by soaking of preformed crystals
Nuclear magnetic resonance	1 000–10 000 as mixtures	Binding affinity together with binding site information through displacement of well characterised compound or HSQC data	2–17 000 (1850)	0.20–0.48 (0.32)	Small to medium-sized libraries
Bioassay (HTFS)	20 000	Binding affinity at specific site or functional activity	0.1–41 000 (2220)	0.21–0.86 (0.50)	No limitation on library size only generic constraints for fragment solubility apply

[a] Data compiled from a review by Alex and Flocco [3••].

to different companies finding for the same target similar or identical fragment starting points through the screening of relatively small fragment libraries of compounds sourced from third-party compound suppliers. Unless there is a divergence in the manner in which the structure-based optimisation is progressed this can lead to companies filing patent applications covering similar chemical matter. A case in point is the cancer target heat shock protein 90 (Hsp90) [37–40], which to our knowledge is being progressed using fragment-based drug discovery by a number of companies including Abbott [41••], Astex and Vernalis [6•]. We initially screened 1200 fragments by a sensitive biochemical confocal fluorescence binding assay for the ATP binding site using a Tamra labelled analogue of geldanamycin. The assay was miniaturised to a 1 μl/well format and conducted on the EVOscreen® Mark II HTS platform using a proprietary 2D-FIDA anisotropy readout ($Z' > 0.8$) with each fragment screened in triplicate at 11 concentrations from 1 to 2000 μM. Forty-five fragments exhibited IC_{50} values between 15 and 1500 μM, and a priority list of fragments was selected for structural studies following review by medicinal chemists. This resulted in 33 fragments being initially selected for structural studies of which co-crystal structures were obtained for 12 of the fragments. We do not know if this 35% success rate from biochemical assay to fragment crystal structure is due to false positives from the screening process or to false negatives from crystallography. Notably, there was no bias for the more potent fragment hits to produce crystal structures or vice versa. This result led us, in subsequent projects, to triage fragment hits by NMR studies as our preferred approach to select compounds for crystallography following biochemical high throughput fragment screening (HTFS). However, we still observe false negatives in the crystallography process. For 4 out of the 12 fragments that were successful in structural studies with Hsp90, we could not obtain the ligand bound structures by soaking, whereas co-crystallisation was successful. We assume that had this target been directly addressed by X-ray crystallographic screening, these fragments would have been missed. In the Abbott Hsp90 programme 11 520 compounds were screened by NMR (2D HSQC), and hits were triaged by orthogonal screening using a fluorescence resonance energy transfer (FRET) biochemical assay before X-ray structure determination of the fragments in complex with

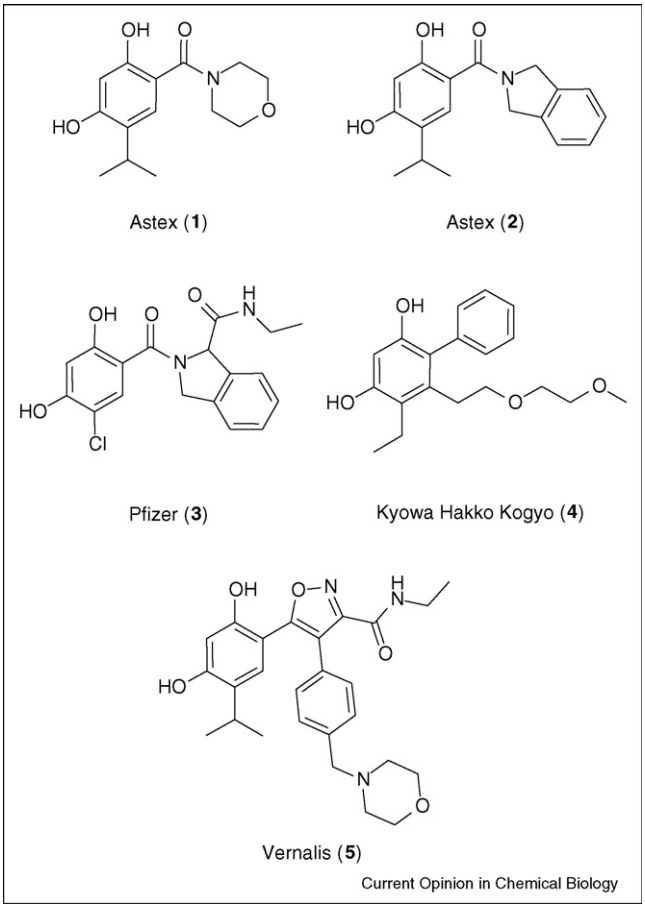
Figure 2



Radicicol, a natural product inhibitor of Hsp90, and three active fragments identified by fragment screening of a 1200 member fragment library that included radicicol-derived compounds. IC_{50} as obtained using a biochemical assay.

Hsp90. The X-ray structures reported by Abbott were all obtained by co-crystallisation. Three of the active fragments that we discovered were related to the natural product Hsp90 inhibitor radicicol (Figure 2). The Vernalis group has independently reported the carboxylic acid of Fragment 2 as an Hsp90 inhibitor [6•], and their X-ray crystal structure of this compound in complex with Hsp90 appears to be similar to the structure that we have solved for Fragment 1 in complex with Hsp90. The resorcinol moiety present in these fragments is a key feature of other Hsp90 inhibitors (Figure 3) such as structurally related compounds from Astex (**1** and **2**) [42,43] and Pfizer (**3**) [44] that we presume were discovered by fragment-based methods. In addition, Kyowa Hakko Kogyo have described resorcinol analogues including compound (**4**) that we presume were derived from SAR studies of the minimal active fragments of radicicol [45], and Vernalis reported compound (**5**) to be derived by structure-based design from an initial HTS hit, although this programme has been presumably assisted by their fragment studies [46]. The fact that so many research groups are focusing on the same starting points for Hsp90 highlights the benefit of having access to either novel fragments not available to others and/or to a large diverse fragment library. This will be particularly the case for well-validated targets for which the structural biology is straightforward such that direct fragment screening by crystallographic methods is possible.

Figure 3

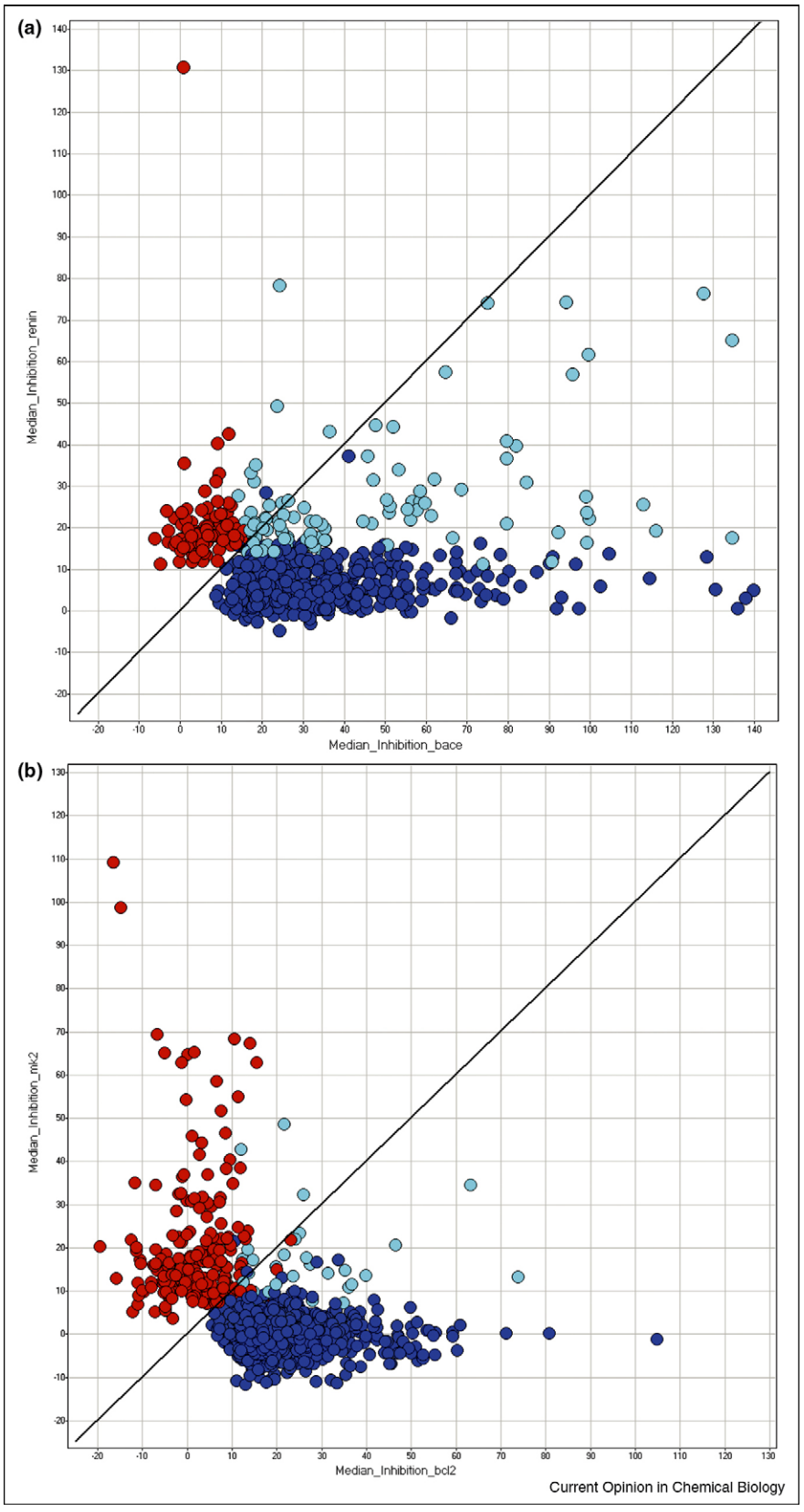


Inhibitors of Hsp90 that feature a resorcinol moiety.

Enriching fragment data sets by parallel screening

For high concentration bioassay based fragment screening we use generic assay formats that allow for a direct comparison of experimental data sets. For instance we use only one or two red-shifted, short-lived fluorescent dyes for ligand or substrate labelling and frequently we develop class-generic assay systems (for proteases, ATPases, kinases, etc.) where only the target of interest and the substrate/ligand is exchanged. This enables the direct comparison of data sets and derivation of valuable information about selectivity and frequent hitters due to fluorescence artefacts or promiscuous fragments. Figure 4 details the outcome of screening of a 20 000 maximum diverse fragment collection (in replicates of $n = 3$) for four prominent pharmaceutical targets. The two aspartyl proteases BACE-1 and renin were screened with a catalytic assay format using fluorescently labelled peptidic substrates at 0.25-fold their respective K_m concentration. The correlation of the median percentage inhibition values at 1 mM fragment concentration (Figure 4a) shows a significant number of statistical fragment hits for BACE-1 but only a few for renin. Interestingly there is a set of dual aspartic acid inhibitors (turquoise circles in Figure 4a) that may be considered of high priority for subsequent ligand structure determination by crystallography. Many of these fragment hits contain head group structural features that facilitate H-bond formation to the catalytic asparates, including primary amine, aminopyrimidine, piperazine and piperdine motifs. Researchers at Plexxikon have applied a similar approach to develop drug-like inhibitors of PDE4 isoforms [33•]. Their workflow comprised screening, using a high concentration HTS technique, a set of 20 000 so called scaffolds (125–350 Da) against a panel of PDE isoforms in order to identify target family scaffolds inhibiting three or more PDE isoforms before developing one common scaffold into nanomolar inhibitors of PDE4 by structure-based drug design. The crucial step in their programme was the identification of a chemical scaffold with a binding mode robust to the introduction of substituents, thus allowing extensive use of *in silico* predictive methods. In Figure 4b we have correlated the median percentage inhibition values for two non-homologous targets, Bcl-2 and the inactive conformation of MAPKAP kinase 2 (MK2). As expected the rich hit populations for these two targets do not significantly overlap, that is, the target-specific fragment hits are *selective*. This implies that there are no promiscuous inhibitors or general artefacts in this sensitive biochemical screening process that would require tedious follow-up work to clean-up the data sets.

Figure 4



Correlation plots of median percentage inhibition values for four pharmaceutical targets screened at Evotec. **(a)** BACE-1 (*X* axis) and Renin (*Y* axis). **(b)** Bcl-2 (*X* axis) and MK2 (*Y* axis). Legend: blue, statistical hit *X* axis; red, statistical hit *Y* axis; turquoise, statistical hit in both screens.

Conclusion

Each fragment screening technique has its advantages; X-ray crystallography provides immediate structural information, NMR provides binding site and affinity information of a very high quality while bioassays provide functionally relevant activity data for larger collections of fragments. However, we and others are seeking to combine the methods in order to maximise their value to fragment-based drug discovery. NMR and biochemical screening of fragments are complementary orthogonal methods that can be used individually or in concert to provide the most effective way of addressing each new biological target of interest. The strength of biochemical screening is that its throughput allows large fragment collections to be screened in a short length of time. This ensures that the most ligand efficient diverse starting points are available for medicinal chemists to select for subsequent optimisation. A further advantage is that screening related targets using generic biochemical assay formats enables insights into target selectivity from the outset. The large number of fragment hits that are obtained through use of biochemical screening of large fragment libraries can be effectively triaged ahead of crystallography by the use of protein NMR. Thus, the most effective way to perform fragment screening is not to rely on a single method but to use orthogonal methods in concert.

Conflict of interest statement

TH and MW are both employees of Evotec, a drug discovery company that applies fragment methods both to its own internal CNS-focused drug discovery and in collaborations with other companies.

Acknowledgements

The authors would like to thank colleagues, in particular, Mark Ashton, John Barker, Mark Brewer, Osamu Ichihara, Pierre Ilouga, Owen Mather, Markus Schade and Dirk Ullmann at Evotec for useful discussions and comments.

References and recommended reading

Papers of particular interest, published within the annual period of the review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. • Jhoti H, Cleasby A, Verdonk M, Williams G: **Fragment-based screening using X-ray crystallography and NMR spectroscopy**. *Curr Opin Chem Biol* 2007, **11**:485-493.
A useful overview of the latest advances in the use of X-ray crystallography and protein NMR to find fragment hit compounds.

2. •• Hajduk PJ, Greer J: **A decade of fragment-based drug design: strategic advances and lessons learned**. *Nat Rev Drug Discov* 2007, **6**:211-219.
In this comprehensive review the authors give a useful perspective of the application of fragment-based drug discovery within the pharmaceutical and biotech industries.

3. •• Alex AA, Flocco MM: **Fragment-based drug discovery: what has it achieved so far?** *Curr Top Med Chem* 2007, **7**:1544-1567.
The authors provide a comprehensive set of examples of fragment hits that have been progressed into lead compounds together with a cheminformatics analysis of both the fragment starting points and the resultant representative lead compounds.

4. Zartler ER, Mo H: **Practical aspects of NMR-based fragment discovery**. *Curr Top Med Chem* 2007, **7**:1568-1581.

5. • Hesterkamp T, Barker J, Davenport A, Whittaker M: **Fragment based drug discovery using fluoresence correlation spectroscopy techniques: challenges and solutions**. *Curr Top Med Chem* 2007, **7**:1582-1591.
This review describes an effective approach to finding fragment hits through biochemical screening and discusses the various orthogonal biophysical methods that can be used to triage fragment hits ahead of structural studies.

6. • Hubbard RE, Davis B, Chen I, Drysdale MJ: **The SeeDs approach: integrating fragments into drug discovery**. *Curr Top Med Chem* 2007, **7**:1568-1581.
An informative account is given of the Vernalis approach to fragment-based drug discovery involving NMR screening followed by X-ray crystallography. This is illustrated by case studies for a number of targets.

7. •• Albert JS, Blomberg N, Breeze AL, Brown AJH, Burrows JN, Edwards PD, Folmer RHA, Geschwindner S, Griffen EJ, Kenny PW *et al.*: **An integrated approach to fragment-based lead generation: philosophy, strategy and case studies from AstraZeneca's drug discovery programmes**. *Curr Top Med Chem* 2007, **7**:1600-1629.
This is a very detailed account of the approach taken by a major pharmaceutical company to apply fragment-based drug discovery approaches to not just structurally tractable targets but also GPCRs.

8. Neumann T, Junker H-D, Schmidt K, Sekul R: **SPR-based fragment screening: advantages and applications**. *Curr Top Med Chem* 2007, **7**:1630-1642.

9. • Wells JA, McClendon CL: **Reaching for high-hanging fruit in drug discovery at protein–protein interfaces**. *Nature* 2007, **450**:1001-1009.
An inspiring overview of recent successes in the discovery of small molecule ligands for difficult protein–protein interaction targets.

10. Hajduk PJ: **SAR by NMR: putting the pieces together**. *Mol Interventions* 2006, **6**:266-272.

11. Congreve M, Carr R, Murray C, Jhoti H: **A 'Rule of Three' for fragment-based lead discovery**. *Drug Discov Today* 2003, **8**:876-877.

12. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ: **Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings**. *Adv Drug Deliv Rev* 1997, **23**:3-25.

13. Siegal G, Eiso AB, Schultz J: **Integration of fragment screening and library design**. *Drug Discov Today* 2007, **12**:1032-1039.

14. Hopkins AL, Groom CR, Alex A: **Ligand efficiency: a useful metric for lead selection**. *Drug Discov Today* 2004, **9**:430-431.

15. Abad-Zapatero C, Metz JT: **Ligand efficiency indices as guideposts for drug discovery**. *Drug Discov Today* 2005, **10**:430-431.

16. Kuntz ID, Chen K, Sharp KA, Kollman PA: **The maximal affinity of ligands**. *Proc Natl Acad Sci U S A* 1999, **96**:9997-10002.

17. •• Leach AR, Hann MM, Burrows JN, Griffen EJ: **Fragment screening: an introduction**. *Mol BioSyst* 2006, **2**:429-446.
This comprehensive review provides valuable insights into the cheminformatic basis for the advantages of fragment screening and the application of fragment methods at GlaxoSmithKline and AstraZeneca.

18. Hajduk P: **Fragment-based drug design: how big is too big?** *J Med Chem* 2006, **49**:6972-6976.

19. Gorse A-D: **Diversity in medicinal chemistry space**. *Curr Top Med Chem* 2006, **6**:3-18.

20. • Blaney J, Nienaber V, Burley SK: **Fragment-based lead discovery and optimization using X-ray crystallography, computational chemistry, and high-throughput organic synthesis**. In *Fragment-Based Approaches in Drug Discovery—Methods and Principles in Medicinal Chemistry*, vol. 34. Edited by Jahnke W, Erlanson DA. Wiley-VCH; 2006:215-248.
This article describes the approach taken at SGX to fragment-based drug discovery involving screening by X-ray crystallography of a fragment library from which analogues can be rapidly prepared by parallel synthesis.

21. Fink T, Bruggesser H, Reymond J-L: **Virtual exploration of the small-molecule chemical universe below 160 Daltons**. *Angew Chem Int Ed* 2005, **44**:1504-1508.

22. Fink T, Reymond J-L: **Virtual exploration of the chemical universe up to 11 atoms of C, N, O, F: assembly of 26.4 million structures (110.9 million stereoisomers) and analysis for new ring systems, stereochemistry, physicochemical properties, compound classes, and drug discovery**. *J Chem Inf Model* 2007, **47**:342-353.

23. Bohacek RS, Martin C, Guida WC: **The art and practice of structure-based drug design: a molecular modelling approach**. *Med Res Rev* 1996, **16**:3-50.

24. • Hann MM, Leach AR, Harper G: **Molecular complexity and its impact on the probability of finding leads for drug discovery**. *J Chem Inf Comput Sci* 2001, **41**:856-864.
This study is part of the theoretical basis of fragment-based drug discovery in that chemical compounds are classified in terms of their complexity and probability of being detected as hits in target-based screening.

25. Barker J, Courtney S, Hesterkamp T, Ullmann D, Whittaker M: **Fragment screening by biochemical assay**. *Expert Opin Drug Discov* 2006, **1**:225-236.

26. Baurin N, Aboul-Ela F, Barril X, Davis B, Drysdale M, Dymock B, Finch H, Fromont C, Richardson C, Simonite H, Hubbard RE: **Design and characterization of libraries of molecular fragments for use in NMR screening against protein targets**. *J Chem Inf Comput Sci* 2004, **44**:2157-2166.

27. • Hubbard RE, Chen I, Davis B: **Informatics and modelling challenges in fragment-based drug discovery**. *Curr Opin Drug Discov Dev* 2007, **10**:289-297.
A useful comparison is given to the different approaches that various companies have taken to building compound libraries for fragment screening.

28. Opera TI, Davies AM, Teague SJ, Leeson PD: **Is there a difference between leads and drugs? A historical perspective**. *J Chem Inf Comput Sci* 2001, **41**:1308-1315.

29. Proudfoot JR: **Drugs, leads, and drug-likeness: an analysis of some recently launched drugs**. *Bioorg Med Chem Lett* 2002, **12**:1647-1650.

30. Nienaber VL, Richardson PL, Klighofer V, Bouska JJ, Giranda VL, Greer J: **Discovering novel ligands for macromolecules using X-ray crystallographic screening**. *Nat Biotechnol* 2000, **18**:1105-1108.

31. Davies TG, van Montfort RLM, Williams G, Jhoti H: **Pyramid: an integrated platform for fragment-based drug discovery**. In *Fragment-Based Approaches in Drug Discovery—Methods and Principles in Medicinal Chemistry*, vol. 34. Edited by Jahnke W, Erlanson DA. Wiley-VCH; 2006:193-214.

32. Hajduk PJ, Huth JR, Fesik SW: **Druggability indices for protein targets derived from NMR-based screening data**. *J Med Chem* 2005, **48**:2518-2525.

33. • Card GL, Blasdel L, England BP, Zhang C, Suzuki Y, Gillette S, Fong D, Ibrahim PN, Artis DR, Bollag G *et al.*: **A family of phosphodiesterase inhibitors discovered by cocrystallography and scaffold-based drug design**. *Nat Biotechnol* 2005, **23**:201-207.
This article describes the application of scaffold-based drug discovery to PDE4 isoforms. The scaffold approach comprises high concentration screening, high throughput co-crystallography and structure-based drug design.

34. Sanders WJ, Nienaber VL, Lerner CG, McCall JO, Merrick SM, Swanson SJ, Harlan JE, Stoll VS, Stamper GF, Betz SF *et al.*: **Discovery of potent inhibitors of dihydroneopterin aldolase using CrystaLEAD high-throughput X-ray crystallographic screening and structure-directed lead optimization**. *J Med Chem* 2004, **47**:1709-1718.

35. Schade M, Oschkinat H: **NMR fragment screening: tackling protein–protein interaction targets**. *Curr Opin Drug Discov Dev* 2005, **8**:365-373.

36. Barker J, Hesterkamp T, Schade M, Whittaker M: **Fragment screening: biochemical versus NMR**. *Innov Pharm Tech* 2007, **23**:19-22.

37. Chiosis G, Vilenchik M, Joungnam K, Solit D: **Hsp90: the vulnerable chaperone**. *Drug Discov Today* 2004, **9**:881-888.

38. Chène P: **ATPases as drug targets: learning from their structure**. *Nat Rev Drug Discov* 2002, **1**:665-673.

39. Beliakoff J, Whitesell L: **Hsp90: an emerging target for breast cancer therapy**. *Anti-Cancer Drugs* 2004, **15**:651-662.

40. Dymock BW, Drysdale MJ, McDonald E, Workman P: **Inhibitors of Hsp90 and other chaperones for the treatment of cancer**. *Expert Opin Ther Pat* 2004, **14**:837-847.

41. •• Huth JR, Park C, Petros AM, Kunzer AR, Wendt MD, Wang X, Lynch CL, Mack JC, Swift KM, Judge RA *et al.*: **Discovery and design of novel Hsp90 inhibitors using multiple fragment-based design strategies**. *Chem Biol Drug Des* 2007, **70**:1-12.
An informative account of the approach taken by Abbott to optimise initial fragment hits at the ATPase site of Hsp90.

42. Chessari G, Congreve MS, Navarro EF, Frederickson M, Murray C, Woolford AJ-A, Carr MG, Rien MA, Woodhead AJ: **Pharmaceutical compounds**. PCT Patent Application; 2006; WO2006109075.

43. Chessari G, Congreve MS, Navarro EF, Frederickson M, Murray C, Woolford AJ-A, Carr MG, Downham R, Rien MA, Phillips TR, Woodhead AJ: **Hydroxybenzamide derivatives and their use as inhibitors of Hsp90**. PCT Patent Application; 2006; WO2006109085.

44. Funk LA, Johnson MC, Kung P-P, Meng JJ, Zhou JZ: **Amide resorcinol compounds**. PCT Patent Application; 2006; WO2006117669.

45. Kitamura Y, Nara S, Nakagawa H, Nakashima T, Soga S, Kajita J, Shiotsu Y, Kanda Y: **Hsp90 family protein inhibitor**. PCT Patent Application; 2005; WO 2005/063222.

46. Brough PA, Aherne W, Barril X, Borgognoni J, Boxall K, Cansfield JE, Cheung K-MJ, Collins I, Davies NGM, Drysdale MJ *et al.*: **5-Diarylisoxazole Hsp90 chaperone inhibitors: potential therapeutic agents for the treatment of cancer**. *J Med Chem* 2008, **4**: published on WEB 11/20/2007.