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Article

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Promiscuous 2-Aminothiazoles (PrATs): A Frequent Hitting Scaffold

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ABSTRACT

We have identified a class of molecules, known as 2-aminothiazoles (2-ATs), as frequent-hitting fragments in biophysical binding assays. This was exemplified by 4-phenylthiazol-2-amine being identified as a hit in 14/14 screens against a diverse range of protein targets, suggesting that this scaffold is a poor starting point for fragment-based drug discovery. This prompted us to analyze this scaffold in the context of an academic fragment library used for fragment-based drug discovery (FBDD) and two larger compound libraries used for high-throughput screening (HTS). This analysis revealed that such "Promiscuous 2-AminoThiazoles" (PrATs) behaved as frequent hitters under both FBDD and HTS settings, although the problem was more pronounced in the fragment-based studies. As 2-ATs are present in known drugs, they cannot necessarily be deemed undesirable, but the combination of their promiscuity and difficulties associated with optimizing them into a lead compound, make them, in our opinion, poor scaffolds for fragment libraries.

INTRODUCTION

Fragment-based drug discovery (FBDD) is becoming a widely-used technique in drug discovery as part of a medicinal chemists arsenal.[1,2] FBDD strategies utilise small molecules (typically ~200 Da), known as 'fragments', that often have relatively low affinities for the target of interest. Nonetheless, numerous FBDD campaigns have demonstrated that it is feasible to elaborate fragment hits to achieve clinically useful compounds.[3] Furthermore, there are published examples where FBDD has enabled the development of potent compounds against targets where HTS of a large library did not yield any useful hits[4] and a review of internal projects by scientists at AstraZeneca revealed that FBDD represents a powerful tool to assess the likelihood of finding highly potent ligands for any given target.[5] This highlights one attraction of FBDD, which is that fragment libraries contain relatively small numbers of compounds (a few hundred to thousands) but are very effective at finding hits because they are able to sample chemical space more efficiently than the larger compounds that are typically found in HTS libraries.[6] One way of illustrating this is to consider that a library of drug-like molecules of 30 heavy atoms might need up to 10^{60} members to efficiently cover chemical space, whereas this number is significantly smaller – around 10^7 – for a library of fragments with around 12 heavy atoms.[7,8] As fragments are smaller, they typically bind with lower affinity to the relevant target, with K_D values ranging from high micromolar to millimolar. In contrast, drug-like hits from HTS typically have K_D values in the high nanomolar to low micromolar range, although in both cases the binding energy per heavy atom (or "ligand efficiency") can be comparable. Fragments can also be developed into lead compounds that are smaller and less lipophilic than those generated from HTS.[9] A highlight of FBDD to date was the development of Vemurafenib, a BRAF kinase inhibitor used for the treatment of late-stage melanoma, which, via medicinal chemistry optimisation, became the first FBDD-derived compound to reach the clinic.[10,11] Since FBDD libraries are usually small, it is essential to ensure that the library is populated with high quality fragments. Some of the key considerations in the design of fragment libraries have been discussed previously.[12,13]

PAINS

A matter of growing concern with screening libraries, for FBDD or otherwise, is the inclusion of compounds that may act promiscuously and display activity both across different target classes and via a number of different assays or biological readouts. These types of compounds, which are widely referred to as Pan Assay INterference CompoundS (PAINS), were first described by Baell and Holloway[14] and are identified by the presence of substructural features that promote frequent-hitting behavior. It has been suggested that they should be excluded from screening libraries for that reason. Whilst in many cases PAINS may appear to give optimizable hits, elaboration often results in flat or confusing structure-activity relationships (SAR).[15] The reasons for their promiscuity are varied and include the presence of potential Michael acceptors, chelation, redox activity, and strong chromophoric interference.[16] Some PAINS scaffolds have easily identifiable problems associated with them, but the chemical basis of the observed promiscuity for many PAINS is unknown. Confounding the complexity of PAINS identification is the fact that there are a small number of cases where seemingly "unprogressable" PAINS have in fact been developed into potent and selective molecules. One example is the PI3Kγ inhibitor (AS-604580) which is based on an alkylidene rhodanine hit containing this known PAINS motif.[17] However, the fact that certain PAINS or PAINS-like motifs are present in some potent and selective molecules does not imply that the PAIN is a viable starting point. In general, the chances that a PAIN will be progressed to a useful lead compound are overwhelmingly smaller than the chances it will not.[16]

There are fourteen sub-classes of 2-ATs that have been categorized as PAINS.[14,16] There are a number of possible reasons for their reported promiscuity, such as their potential photoreactivity or the presence of impurities generated by their chemical precursors, for example where 2-ATs are prepared from bromomethylketones.[14] 2-ATs have also been identified as potentially thiol-reactive, which is another mechanism that produces problematic screening hits. For example a subclass of 2-

ATs was identified in the ALARM-NMR assay as being thiol-reactive,[18,19] although in this case it is possible that their reactive precursors were responsible.[20]

That 2-ATs can be progressed to generate useful compounds is testified by a number of marketed drugs, including antibiotics such as carumonam, cefcapene, cefdinir, cefditoren, cefepime, cefetamet, cefoselis, cefotaxime, cefotiam, cefpodoxime, cefpirome, ceftazidime, ceftibuten, ceftriaxone; talipexole and pramipexole, dopamine agonists for the treatment of Parkinson's disease; mirabegron, a β_3-adrenoceptor agonist used to treat overactive bladder; and riluzole, a 2-aminobenzothiazole used to treat acute myeloid leukaemia (AML). Conversely, 2-ATs have displayed cytotoxicity and metabolic instability as antimycobacterial and antiplasmodial agents.[21] Thus, the value of 2-ATs in screening collections is currently unclear. Herein, we describe our efforts to determine if 2-ATs are promiscuous binders by both FBDD and HTS techniques.

RESULTS

FBDD Screening

FBDD screens were undertaken at the Monash Institute of Pharmaceutical Sciences (MIPS) using a library of 1137 fragments comprising molecules that pass both biophysical and chemical filters, are chemically and structurally diverse, are soluble at 1 mM in aqueous buffer, and can be chemically elaborated from readily accessible precursors.[12,22] Chemical filters that were applied in assembling the fragment library include the removal of PAINS,[14] unwanted functionality[13] and reactive groups.[23] A review of 14 fragment screening campaigns with this library, in which the primary readout of fragment binding was saturation transfer difference nuclear magnetic resonance spectroscopy (STD-NMR),[24] revealed that at least one 2-AT from the library had been identified as a hit in every case.[12] This led to our investigation of their role as potential promiscuous binders using an orthogonal biophysical technique. We report here the results of our study and analysis of all 2-ATs in our fragment library (Figure 1) undertaken using surface plasmon resonance (SPR)

against six different protein targets. Target proteins were the *Plasmodium falciparum* apical membrane antigen AMA1,[22] the E3 ubiquitin ligase adapter protein SPSB2,[25] two DsbA oxidoreductases from different bacterial species (oxidoreductase 1 and 2), carbonic anhydrase II, and a kinase. These proteins were selected as they exhibit diversity in function and have little structural similarity across their binding sites. All except oxidoreductase 2 had known positive controls that were used in the SPR assays. In addition, the fragment screen contained a number of internal controls. Four fragments used in this study were identified previously as binders to SPSB2 by SPR (**1**, **3**, **15**, **17**; our unpublished data), carbonic anhydrase II was expected to show a very strong preference for compounds containing a terminal sulfonamide and a prior fragment-screen of oxidoreductase 2 (our unpublished data) had revealed only very weak binding for any fragment. Thus, oxidoreductase 2 served as a negative control.

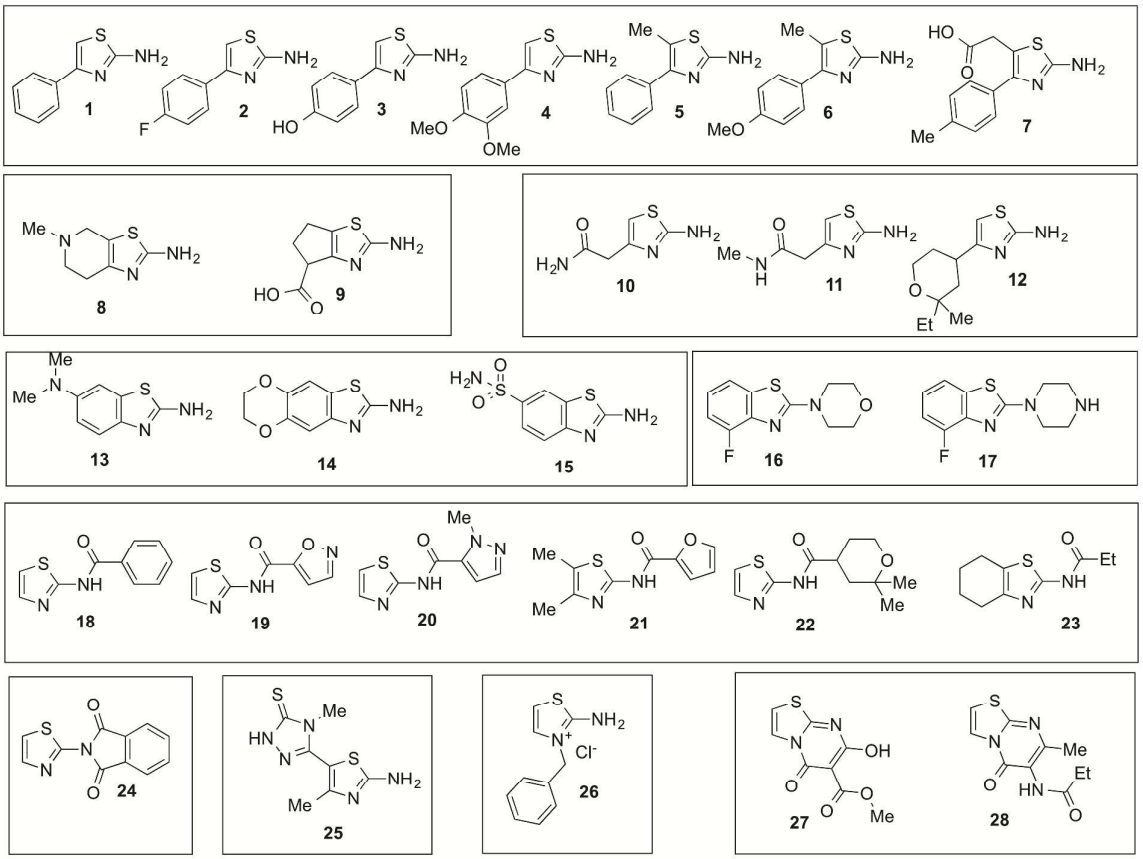


Figure 1. 2-Aminothiazole (2-AT) and 2-AT-like compounds in the MIPS fragment library[12]

Dose-response determinations were undertaken for positive controls (available for all proteins except oxidoreductase 2) and gave K_D values that were within the expected ranges for each target protein (Supplementary Figures S1 and S2), confirming that all proteins were stable and active on the sensor chip under the conditions used. Binding of 2-ATs was carried out at a single concentration (200 μM) in duplicate, and the average raw response was converted to percentage occupancy of binding, corrected for the molecular weight of each compound (Figure 2). The sensorgrams for selected 2-ATs can be found in Supplementary Figures S3–5. Injections of buffer blank were included between each compound, both to provide for double referencing and to minimise carry-over problems from poorly-behaved compounds.

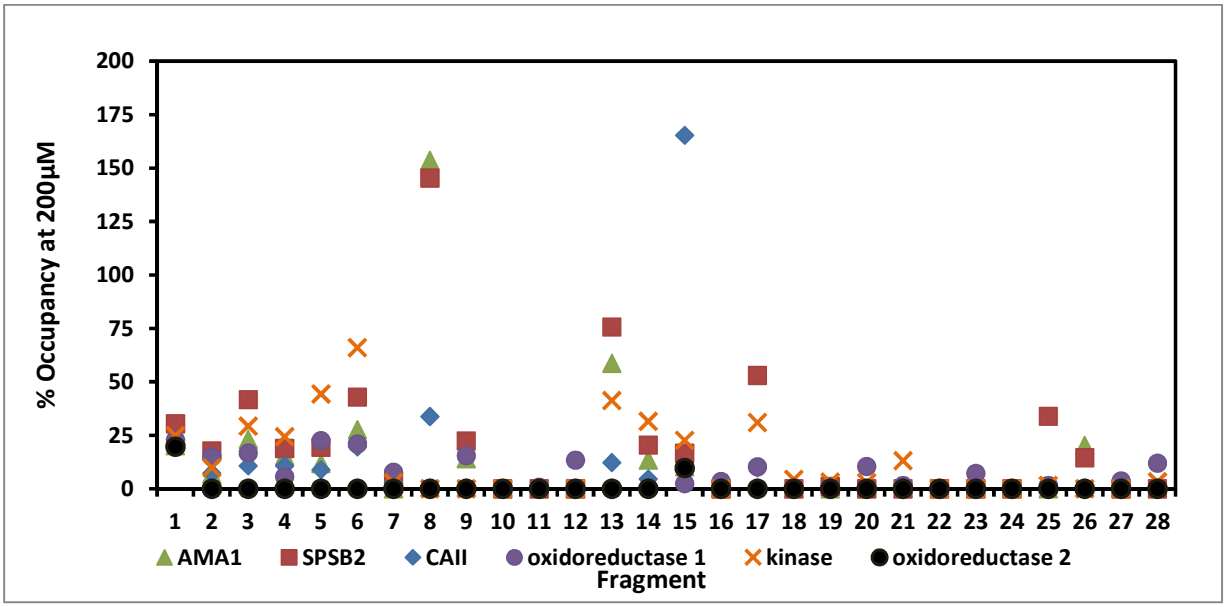


Figure 2. Binding of 2-ATs to AMA1, SPSB2, CAII, oxidoreductase 1, a kinase and oxidoreductase 2. Compounds were tested at a concentration of 200 μM, and the raw response converted to percentage occupancy.

Figure 2 reveals a consistency in the pattern of protein binding. Only two 2-ATs (**1** and **15**) demonstrated any binding to oxidoreductase 2, which is consistent with previous observations that this protein has very limited capacity to bind small molecules. For the other five proteins, there were two cases (fragment **8** and **15**) where a large range in the fractional occupancy was observed,

ranging from ~0% to > 150%. Otherwise, most fragments showed little discrimination in binding, with fragments typically binding to either all five proteins or none at all. While there were some exceptions, there was also a general tendency for fragments to bind each of the five proteins at similar occupancy, suggesting approximately the same affinity and thus providing very little clear SAR.

To examine the SAR in a more systematic manner, 2-ATs from the MIPS library were clustered based on linear fingerprints and key chemical features, and the screening results examined for each grouping. Compounds **1** – **7** feature 4-aryl substitutions with a free 2-amino group, with or without a substituent at the 5-position. Fragments **8** – **12** maintain the free 2-amino group, with **8** and **9** containing fused aliphatic rings, whereas **10** – **12** have aliphatic substituents at the 4-position. Fragments **13** – **15** integrate benzothiazoles with their 2-amino group intact, while fragments **16** and **17** have morpholino or piperazino attachments through their 2-amino group, respectively. Fragments **18** – **24** are 2-amido containing thiazoles and **27** and **28** are thiazolo[3,2-*a*]pyrimidines, in which the 2-amino group is incorporated in the heterocycle. The triazolothione (**25**) and thiazolium (**26**) complete the selection of 2-ATs from the MIPS library. These molecules contain diverse substituents around the 2-AT core. Thus, despite the chemical diversity in the 28 2-ATs in the library, evaluation of their binding to the different targets provided little clear SAR.

As an example, 4-phenylthiazol-2-amine (**1**) highlights the problem with this class of molecule, showing binding to all six proteins examined, all with similar occupancy levels (~25%). As this fragment contains 12 heavy atoms this level of binding corresponds to a favorable ligand efficiency (i.e. binding energy per heavy atom) in each case.[26] Thus, if this fragment were tested in an isolated setting against one particular protein, the likelihood of follow up by medicinal chemists would be high, demonstrating the insidious behavior associated with this class. To rule out an impurity in the commercial preparation of fragment **1**, it was resynthesised and purified. This gave similar

occupancy levels to the commercial product across all protein targets, indicating that the problem is inherent to the molecule itself, as opposed to reactive precursors or side-products potentially present in the commercial source. Furthermore, the initial STD-NMR screening data demonstrated binding of **1** rather than precursors or side-products and also revealed that the binding was non-covalent (Supplementary Figure S6). This rules out several of the potential mechanisms for promiscuity, including photoreactivity, thiol reactivity and the presence of reactive precursors.

Fragments **2** – **6** show binding to all of the proteins examined except oxidoreductase 2. The addition of the 5-methyl group appears to have little effect, as does the nature of the substituents on the 4-aryl ring, which include hydroxy, methoxy and fluoro moieties. The addition of carboxylic or amido containing chains (**7**, **10**, **11**) at the 4- or 5-position, coupled with saturation of the 4-aryl ring (**12**), seems to abrogate promiscuity, although, of these fragments, only **12** is observed to bind to any of the proteins at an occupancy > 10% and **12** binds only to oxidoreductase 1. Of the benzothiazole fragments with a 2-amino group, **13** does not bind CAII or oxidoreductase 2, whereas **14** does not bind to oxidoreductase 1 or 2. Fragment **8** binds to three proteins, however occupancy levels > 100% of the theoretical R_{max} are observed for two proteins (AMA1 and SPSB2). Fragment **15** is the only sulfonamide in the series and demonstrates affinity to CAII, as expected for this protein since it specifically binds terminal sulfonamides, but several other fragments (for example **3**, **8**, **13**) also demonstrate binding to CAII.[27] The 2-substituted aminothiazoles are an interesting test case; morpholino (**16**) shows little to no binding to any protein tested, whereas piperazino (**17**) binds to five of the six proteins. The 2-amido and heterocyclic thiazoles (**18** – **24**, **27** and **28**) show little or no binding to any of the proteins and do not appear to be inherently promiscuous. Triazolo thione **25** and thiazolium **26** showed no binding to five and four of the targets, respectively. In summary, 2-ATs appear to be generally promiscuous at fragment screening concentrations whereas the corresponding amides are not. Within the set of 2-ATs, there are no clear characteristics that

distinguish the more promiscuous binders from their less promiscuous counterparts, and thus there is no clear SAR.

In order to extend the SPR a sample of 2-ATs were evaluated for binding to oxidoreductase 1 using 2D heteronuclear single quantum correlation (HSQC-NMR) studies. Oxidoreductase 1 was chosen for this analysis as it is relatively small (21 kDa), expressed at high levels in bacteria and amenable to isotope labeling and analysis by protein-detected NMR. Four of the fragments (**1**, **5**, **6** and **14**) were tested for binding to oxidoreductase 1 by recording ^{1}H-^{15}N HSQC NMR in the presence and absence of each fragment. Binding was analysed by measuring chemical shift perturbations (CSPs) in the spectrum of the protein upon addition of the fragment (Figure 3).

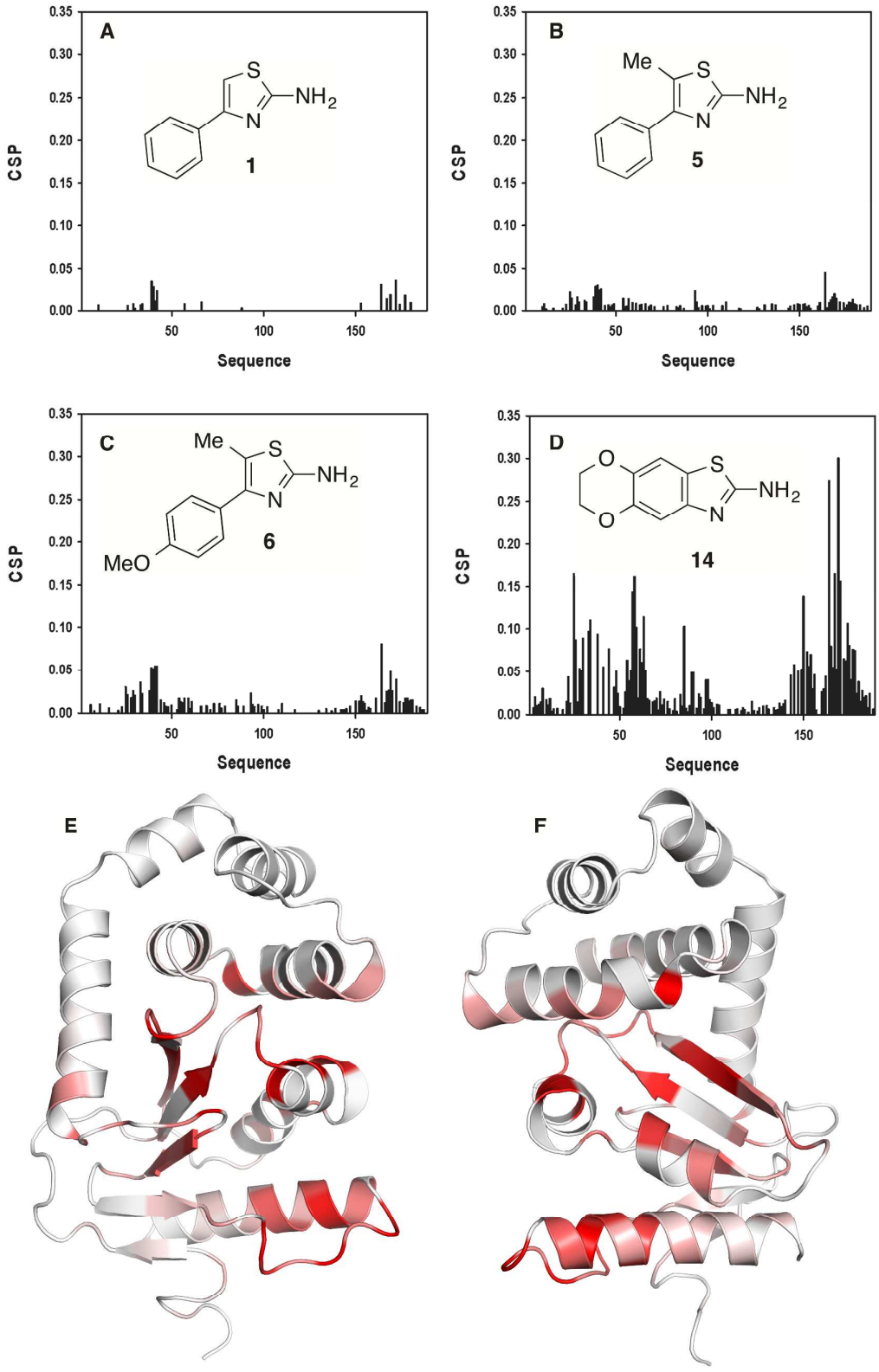


Figure 3. (A-D) HSQC CSPs versus sequence of oxidoreductase 1 at 125 μM with fragment concentrations at 1 mM for **1**, **5**, **6** and 1.5 mM for **14**. Front (E) and back (F) views showing the extent of CSPs of 14 with oxidoreductase 1 (dark red to light red).

The SPR data revealed that **1**, **5** and **6** showed similar levels of binding to the protein, whereas no binding was observed by SPR for **14**. In contrast, the extent of CSP observed in the NMR data suggested that whilst **1**, **5** and **6** showed similar extents of CSP, fragment **14** showed the largest CSP in the spectra. This would normally suggest that **14** is the fragment that binds to oxidoreductase 1 with the highest affinity. However, analysis of the location and extent of CSP on the structure of oxidoreductase 1 (Figure 3E and 3F) revealed clusters of strong CSP at distinct sites on the protein, and a concentration dependence of the CSP that was inconsistent with 1:1 binding stoichiometry (Supplementary Figure S7). Taken together the NMR data suggest that **14** binds to oxidoreductase 1 at more than one site. For fragments **1**, **5** and **6**, the smaller CSP observed make it difficult to determine whether they are binding at more than one site.

Protein-detected NMR was also used to investigate the binding of 2-ATs to a second test protein SPSB2. In this case, ^{19}F-NMR studies on 5-F-Trp-SPSB2 have proven effective as an analytical tool for determining binding to the active site of the protein.[28] Specific binding of peptides and ligands to the functionally important binding site is characterised by a substantial downfield shift of the fluorine resonance corresponding to Trp207 (Figure 4). In contrast, no downfield shift of this peak was observed for the 2-ATs that were observed to bind SPSB2 by SPR, despite their reasonable binding occupancy at 200 μM. This suggests that these 2-ATs do not bind to the active site of SPSB2, and that binding probably occurs at a secondary site on the protein. The ability to bind to more than one site on a protein may underpin the promiscuity that we have observed with some 2-ATs.

We have previously reported analysis of binding of a small set of 2-ATs to AMA1.[22] In the case of AMA1, 4-phenylthiazol-2-amine (**1**) was identified as a hit in the primary screen and a series of 4-aryl substituted 2-ATs was synthesised to generate preliminary SAR. The structures and percentage occupancy data of an extended series of 4-aryl substituted 2-AT analogues and their binding to

AMA1 as determined by SPR are presented in Supplementary Figures S8 and S9. All of the analogues were found to bind with low affinity to AMA1 and no clear SAR was derived from the series.

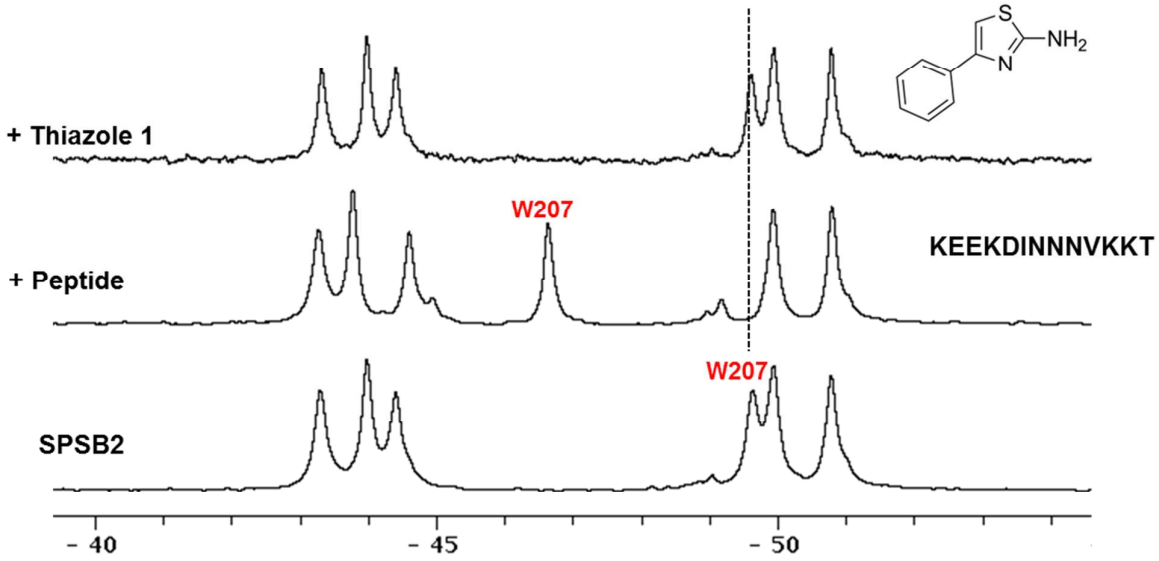


Figure 4. ^{19}F-NMR spectra of 5-F-Trp-SPSB2 alone (bottom), with 500 μM control peptide (middle), and 3 mM **1** (top). Specific binding of the control peptide to the active site is characterised by a downfield shift of the peak corresponding to Trp207.[25] No such shift is observed for the thiazole fragment, suggesting a different mode of binding to SPSB2.

HTS Screening – Academic and Corporate Collections

We were interested in assessing the apparent promiscuity of 2-AT derivatives in fragment-based *vs* high-throughput screening. To analyse the latter, we adopted two approaches. The first was to analyse the relative prevalence of 2-AT-based PAINS in a HTS library of 93,000 compounds housed at the Walter and Eliza Hall Institute (WEHI).[29] The second was to analyse the full AstraZeneca HTS screening deck. With respect to the first approach, a search of the WEHI HTS library revealed 989 2-AT-based compounds and an additional 1012 compounds where the amine was acylated. Amongst these are 14 sub-classes of PAINS, the structural definitions of which are

given in Supplementary Figure S10. This analysis was concordant with data from our fragment library in that no PAINS class contains 2-ATs in which the 2-amino group is acylated.

The incidence of frequent hitting 2-ATs was then investigated with greater statistical power using a large corporate collection (AstraZeneca, January 2014). These compounds have been tested in a range of HTS campaigns with concentrations typically around 10 μM. To assess the promiscuity of 2-ATs we employed a descriptor that has been designed to flag potential frequent-hitter behavior for all compounds with sufficient data.[30] This descriptor is calculated by first designating a compound as active/inactive in each available screen using data from all historical AstraZeneca HTS campaigns. The body of HTS data that is available varies from compound to compound for a variety of reasons (age of compound, sample availability, membership of screen sets, manual collation), so potentially anomalous binding behavior was designated by comparing observed incidence of activity for a given compound to the expected activity for an 'average' compound. In this way a descriptor (termed the pBSF score) was derived that indicates whether the compound is more active than expected based on historical observations.[30] This knowledge-based descriptor is calculated as the negative logarithm of the probability of encountering the observed level of activity for a compound that behaves normally by chance. A low probability, and hence a high score, indicates that it is very unlikely that the observed level of activity would be observed for a well-behaved compound, which therefore suggests that the compound is a promiscuous hitter. We first analysed the number of 2-ATs that had a pBSF score above a threshold score of 2.0. Secondly, we divided the 2-ATs into classes based on substructures, and determined the proportion of each substructure that had pBSF scores > 2.0.

A comparison was made of the pBSF score for the 61,040 2-ATs within the AstraZeneca collection. For a visual comparison, a set of the same size was selected at random from the library, as documented in Figure 5. For the 2-ATs there were 6,122 compounds with a score > 2 (incidence of

10.0%), whereas a randomly picked set of the same size has, on average, 3953 ± 61 compounds with a score > 2 (incidence of 6.5% ± 0.1%, as determined by a bootstrapping analysis). It is evident that the incidence of frequent hitters in the random set is significantly lower than for the 2-ATs (confidence P>99.9%, as derived from bootstrapping analysis), which suggests that the 2-ATs show a tendency to be promiscuous that is significantly higher than seen in a diverse set of randomly-selected compounds.

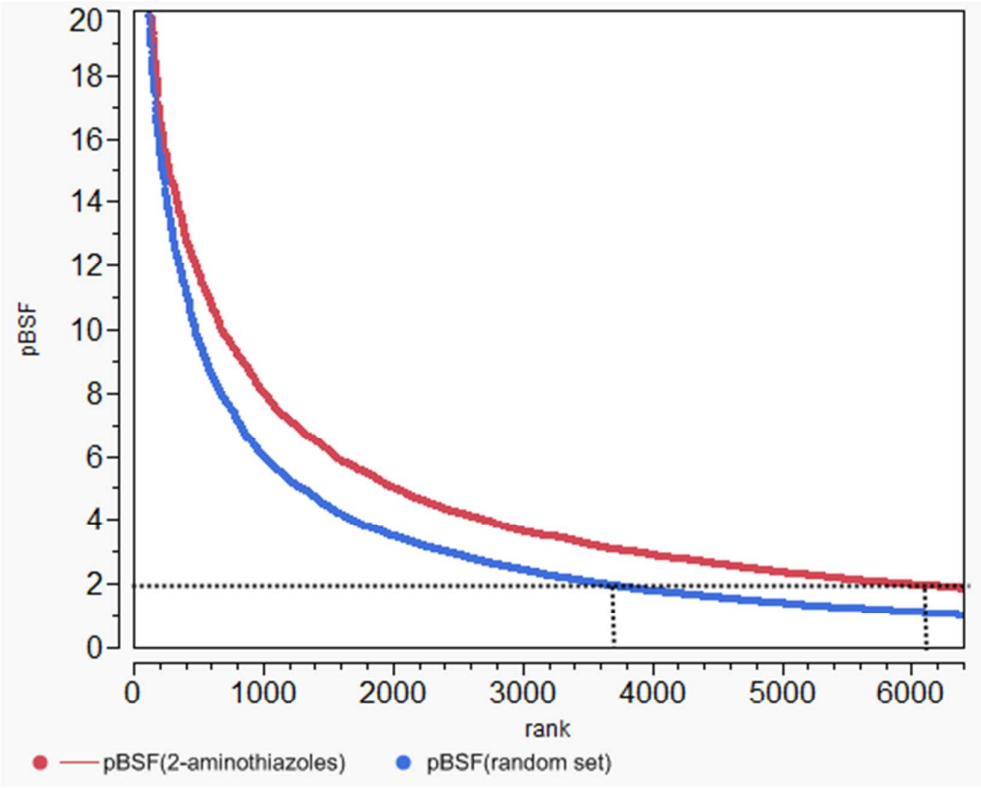
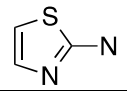
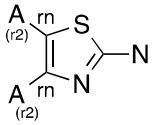
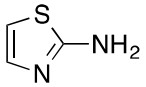
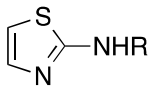
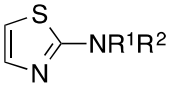
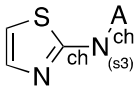
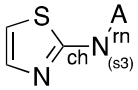
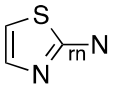
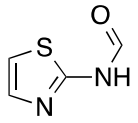


Figure 5. Rank-ordered frequent hitter scores for the first 6,122 2-ATs (red line) from a total set of 61,040 2-ATs retrieved by substructure from the AstraZeneca screening collection. Dotted lines show the numbers of compounds at a threshold value > 2, which is indicative of anomalous binding behavior.

We then divided the 2-AT structural classes using the substructures shown in Table 1 and counted the incidence of suspicious compounds using a threshold of > 2 to distinguish potential frequent hitters from 'clean' compounds and those lacking sufficient data.

Table 1. Nine simplified 2-AT classes were inspected for promiscuous behavior across the AstraZeneca corporate collection. Counts are shown for subsets of small molecules (MW < 300) and larger compounds (MW ≥ 300).

Class		Substructure [a]	N	N with data MW <300	N with data MW >=300	N(FH) MW <300	N(FH) MW >=300	fraction pBSF>2 LowMW	fraction pBSF>2 highMW
1	*aminothiazoles*		77,826	8,070	52,970	559	5,563	6.93	10.50
2	*fused aminothiazoles*		3,259	284	2,190	17	144	5.99	6.58
3	*primary amines*		8,678	1,781	4,425	143	564	8.03	12.75
4	*secondary amines*		55,445	5,336	38,337	372	3,970	6.97	10.36
5	*tertiary amines*		13,864	978	10,312	53	1,068	5.42	10.36
6	*tertiary amines, acyclic*		3,517	304	2.795	15	108	4.93	3.86
7	*tertiary amines, cyclic*		10,307	661	7,495	37	960	5.60	12.81
8	*thiazole cyclic amines ('embedded')*		59	21	25	0	0	0.00	0.00
9	*acylated thiazole amines*		29,816	2,613	21,447	99	1,657	3.79	7.73

[a] Structure legend: A, any atom; rn, ring bond; ch, chain bond; s*n,* substitution count *n*; r*n*, number of ring bonds

The data in Table 1 are consistent with the patterns observed with the MIPS FBDD and WEHI HTS data, namely that 2-ATs show a higher incidence of anomalous binding behavior across a number

of substructure classes. Typically, structures across the AstraZeneca collection that have on average ≤ 6.0% of the compounds within the class with a pBSF score > 2.0 are not considered to be frequent hitters. Here, an elevated incidence (approx. 10%) of such behavior can be seen for the overall class of 2-ATs and for subclasses 1, 3, 4, 5 and 7, which is in line with observations for the WEHI HTS library.

It is notable that promiscuity can be observed in the HTS data even within sets of low-MW compounds For example, within class 3, there are 1781 primary amines with MW < 300, of which 8% are classified as frequent hitters. Although these compounds are "fragment-like" in terms of their size, they are observed to be promiscuous in the HTS data despite being screened at a typical HTS concentration of 10 μM, which suggests that their promiscuity in the FBDD data is not due solely to the higher concentrations used in the fragment screen.

Subclass 9 comprises the acylated 2-ATs, which show a lower incidence of frequent-hitter behaviour than the entire class of 2-ATs both for the low-MW and high-MW set. Nonetheless, the incidence of frequent-hitter behavior in the high-MW set is somewhat higher than the expected "normal" level of 6.0%, although this can be explained by biases that are apparent in target-specific analyses of the screening data. Inspection of a target-specific frequent hitter descriptor (results not shown) reveals that subclasses 3, 4 and 9 show an increased incidence of kinase activity. As kinases are typical drug targets pursued by pharma companies, a bias may be observed in the data where kinase-like motifs have been screened preferentially in kinase-targeted screens. This skews the descriptor results based on such data to some extent, i.e. some of the frequent hitters identified may actually be kinase-frequent hitters, and not necessarily problematic. Within the 1,657 potential frequent hitters of kinase-like class 9 (MW≥300), a subset of 556 molecules appears to be kinase-frequent hitters. Subclass 7 contains a structural motif less likely to hit kinases, yet appears to have a high frequent-hitter incidence and contains 960 suspicious compounds (MW≥300). Of these, a

smaller fraction of only 136 display kinase-related frequent hitter behavior, suggesting that the origin of frequent-hitting behavior in the remaining compounds is mostly not related to kinases. We suggest therefore that the somewhat increased incidence of frequent hitters we observe for the acylated 2-ATs in this data set is an artefact resulting from a kinase-activity bias, and that the acylated 2-ATs as a whole do not meet the criteria to be categorised as frequent hitters.

Using the AstraZeneca 2-AT set of 61,040 compounds and data derived from the AstraZeneca corporate collection, we investigated overall correlations of frequent hitter incidence with lipophilicity (experimental logD, clogP), experimental solubility (pSol, calculated as log[Sol/uM]), calculated polar and non-polar surface area (PSA and NPSA, in $Å^2$ and %PSA in %), and molecular volume.[31,32] Correlations are observed with ion class, PSA, %PSA, as well as donor and acceptor counts (Supplementary Figure S11), suggesting that there could be a relation with polarity (as each of these properties reflects polarity and they are interrelated). A weak trend with logD (experimental octanol-water partitioning coefficient) was observed, with low-logD compounds somewhat more likely to be frequent hitters. No relation is observed with experimental aqueous solubility, but a trend is evident with increasing clogP. Although there are no categorical reasons for promiscuity in these compounds, the observation that ionisable groups and high lipophilicity may increase anomalous behavior in the 2-AT class is in line with recent observations by Tarcsay and Keserű,[33] who observed similar trends.

DISCUSSION

We have demonstrated that certain members of the class of compounds containing a 2-AT substructure are frequent hitting and promiscuous fragments in the context of FBDD, where screening is undertaken using biophysical binding assays. We have dubbed these fragments Promiscuous 2-AminoThiazoles (PrATs). Analysis of binding of the 28 2-ATs in the MIPS fragment library was undertaken by SPR and NMR against six unrelated protein targets. Although some patterns have emerged, such as the presence of the free 2-amino group seeming to promote

promiscuity, a clear mechanism of action has not been identified at this point. Nonetheless, our SPR analysis produced flat and confusing SAR against several of the targets, which has previously been noted in the characterisation of PAINS identified in HTS.

As several approved drugs contain a 2-AT, it is clearly possible to accommodate this structure in a therapeutically useful compound. However, it is noteworthy that a number of PAINS subclasses also contain the 2-AT chemotype, which suggests that the 2-AT may carry some risks if selected for development. For example, in common with many aromatic amines, 2-ATs can be Ames positive dependent on the substitution pattern. Further, aminothiazoles have been associated with liver toxicity via bioactivation of the double bond, leading to formation of thioureas that are further bioactivated. Problems relating to reactivity and covalent modification, however, are unlikely to contribute to the observed promiscuity in biophysical binding assays, as the ligand-detected NMR assays used in FBDD provide evidence of both compound identity and binding simultaneously, whilst both NMR and SPR discriminate between covalent and non-covalent interactions. Therefore the FBDD data suggest that the binding observed is non-covalent and can be attributed to the 2-ATs themselves rather than reactive precursors or impurities in the samples.

Analysis of the AstraZeneca HTS data reveals that 2-ATs show elevated frequency as screening hits relative to the compound library as a whole, although the percentage (10%) is significantly lower than the corresponding value for other known PAINS classes, which can be around 15-20%.[30] The AstraZeneca data also suggest that acylation of the 2-amino group ameliorates the problem of frequent hitting, which indicates that not all 2-ATs are problematic in the context of HTS. Similarly, only 3.2% of 2-AT-containing compounds in the WEHI HTS library are defined as PAINS, which does not provide a strong case for exclusion of all 2-ATs from HTS collections.[14] Thus, the two HTS analyses are in broad agreement with each other and suggest that the majority of 2-ATs do not seem to be promiscuous at HTS-relevant concentrations in the 10 – 25 μM range.

Even at HTS-relevant concentrations, some optimizable hits can be relatively promiscuous and we have previously identified a 2-aminobenzothiazole derivative[16] that hits four out of the six HTS assays selected for the PAINS analysis,[14] yet led to a highly selective and potent compound.[34]

Nonetheless, many 2-ATs were observed to be problematic in both HTS and FBDD, suggesting that the promiscuity observed in FBDD is not due solely to the higher concentration used in the fragment screens. Whilst the higher hit rates that are expected in FBDD dictate that many fragments are likely to hit more than one target, these hits are only useful if they can be elaborated into more potent compounds. On this basis, it is possible that certain substructures such as 2-ATs, which show some limited promiscuity in HTS assays, but would not be flagged for exclusion from a fragment library by analysis of chemical or physical properties, are in fact undesirable as members of a fragment library based on a retrospective analysis of their behaviour in screening assays. A similar approach has been described previously in the HTS setting for deprioritising compounds that are known to be promiscuous.[35]

CONCLUSION

We have identified 2-ATs as a promiscuous substructure in screens of our fragment library. Each of the 28 fragments containing a 2-AT substructure passed all of the biophysical and chemical filters that were used in designing the library and were demonstrated to have appropriate purity and aqueous solubility. However, based on our findings reported here and our unsuccessful attempts to optimize these fragments against different targets, we have removed 2-ATs from the fragment library at MIPS, on the basis that we judge the prospects for such compounds to be progressable as much smaller than the chances that they will not.

EXPERIMENTAL SECTION

General procedures. Biacore sensor chips, *N*-hydroxysuccinimide (NHS), *N*-ethyl-*N*′-(3-dimethylaminopropyl) carbodiimide (EDC), ethanolamine HCl, HBS-P running buffer, and P-20 surfactant were obtained from GE Healthcare. Carbonic anhydrase II and 4-carboxybenzyl sulfonamide were obtained from Sigma-Aldrich. Isabelle Lucet (WEHI) provided the kinase protein and positive control H4. Doubly His-tagged oxidoreductases 1 and 2 were provided from Martin Williams (MIPS). All 2-AT fragments were obtained from Chembridge or Life. The aqueous solubility, identity and purity (>95%) of the purchased compounds was verified by analysis of ^{1}H-1D NMR spectra as described previously.[12]

Surface Plasmon Resonance (SPR) conditions

Expression and purification of hexahistidine-tagged AMA1,[36] and GST-tagged SPSB2,[25] were as described previously. Binding of 2-ATs to the target proteins was measured on a Biacore T200 instrument (GE Healthcare). AMA1, SPSB2, and CAII were immobilized onto a CM5 sensor chip (Biacore) by standard amine coupling chemistry using sodium acetate at pH 4.5, in running buffer A. Doubly-His_6-tagged oxidoreductase proteins were immobilised onto an NTA chip (Biacore) charged with Ni^{2+} according to manufacturer's instructions, in running buffer B. Singly-His_6-tagged Kinase was immobilized to an NTA sensor chip charged with Ni^{2+} using the Capture-Couple method,[37] in running buffer C. Immobilisation levels were typically 9000 RU for AMA1, 6000 RU for SPSB2 and CAII, and 2000 – 3000 RU for both oxidoreductases, and the kinase.

Running buffer A: 25 mM HEPES, 150 mM NaCl, 5% DMSO, 0.005% P-20, pH 7.4.

Running buffer B: 25 mM HEPES, 200 mM NaCl, 5% DMSO, 0.005% Tween-20, pH 8.0

Running buffer C: 25 mM HEPES, 150 mM NaCl, 4 mM $MgCl_2$, 2 mM TCEP, 3% DMSO, 0.005% Tween-20, pH 7.5.

SPR Screening of 2-ATs

Binding assays were carried out at 25°C using a flow-rate of 100 µL/min in running buffer A (AMA1, SPSB2, CAII), running buffer B (oxidoreductase 1 and 2), or running buffer C (kinase).

200 mM fragment stocks were diluted in the appropriate running buffer to obtain 200 μM working concentrations. The association and dissociation phases of binding were each followed for 30 s. An identical injection of running buffer was carried out between each fragment injection, and the average of adjacent blanks was subtracted from the raw fragment response. Raw sensorgram data were reduced, solvent-corrected, and double-referenced using BIAEvaluation Software (GE Healthcare). Raw responses were converted to percentage occupancy by the method of Giannetti[38] using an R_{max} value based on the response measured with a saturating concentration of the appropriate control, and the molecular weight of each fragment. For oxidoreductase 2, there is no positive control available, and binding is reported as percentage of the theoretical Rmax.

SPR Positive Controls

The R1 peptide[39] was used as a control for AMA1. A peptide corresponding to residues within the N-terminus of inducible nitric oxide synthase was used as a control for SPSB2.[25] 4-Carboxybenzyl sulfonamide was used as a control for CAII. An elaborated fragment "H4" was used as a control for the kinase. An elaborated fragment "LA010" (our unpublished data) was used as a control for oxidoreductase 1. As oxidoreductase 2 has demonstrated a weak ability to bind fragments (unpublished data), no positive control was available for this protein. Dose-response determinations were carried out for all positive controls to confirm protein activity. A concentration series of 2-fold dilutions in running buffer was used, with a top concentration of 1 μM (R1), 5 μM (iNOS peptide), 10 μM (4-CBS), 20 μM (H4) or 200 μM (LA010) (see Supplementary Figure S1).

Screening by STD-NMR

Screening was performed by recording STD-NMR experiments on cocktails of up to six fragments in the presence of the target protein (1-10 μM). Screens were undertaken at 600 MHz or 800 MHz on spectrometers equipped with a cryogenically cooled probes. Where possible, screens were carried out in phosphate buffer prepared in > 90% $^{2}H_2O$, to minimise spectral interference from either buffer signals or the $^{1}H_2O$ resonance, and at a pH close to 7.0 and temperature of 10 °C. Other buffer conditions were used where necessary as dictated by the stability of the target protein.

The resulting data were processed in Topspin (Bruker Biospin) and analysed manually. STD signal intensities in the spectra were qualitatively classified as strong, medium or weak based on the relative intensity of signals observed in the low-field region (> 5.0 ppm). Relative intensities were based on the most intense STD signal (I_{max}) identified across all the STD spectra for a particular target. A positive STD signal was categorised as strong where the intensity was > 50% I_{max}, medium where the intensity was > 25% I_{max} and < 50% I_{max} or weak where the intensity was < 25% I_{max}. If the fragment contained no resonances in the low-field region of the ^{1}H-NMR spectrum, the aliphatic region (< 4.5 ppm) was analysed and such fragments were considered hits if any positive STD signal was observed. No attempt was made to categorise aliphatic STD signals due to the potential for either direct excitation by the on-resonance saturating pulse in the STD experiment and/or interference from overlapping protein resonances (See Supplementary Figure S6).

Analysis of binding by HSQC-NMR

To analyze the location of the fragment binding site on oxidoreductase 1, 2D ^{1}H-^{15}N HSQC NMR spectra were recorded in the absence and presence of fragments **1**, **5**, **6**, and **14**. A reference ^{1}H-^{15}N HSQC of the protein (125 μM [U-^{15}N]-labeled protein; 2% DMSO-d_6; 50 mM HEPES; 50 mM NaCl; pH 6.8) was acquired and compared with a spectrum acquired under the same conditions for a sample containing the oxidized oxidoreductase 1 in the presence of each fragment (125 μM [U-^{15}N]-labeled protein; 1-1.5 mM fragment; 1-2% DMSO-d_6; 50 mM HEPES; 50 mM NaCl; pH 6.8). Subsequently, the protein was titrated with **14** (0.09-1.5 mM). 3 mm thick walled tubes of sample volume ~ 160 μL were used for NMR data collection. All data were acquired on a Bruker 600 MHz spectrometer equipped with auto-sampler and CryoProbe at 300K. Standard acquisition and processing parameters were used throughout (See Supplementary Figure S7).

^{19}F NMR Studies of 5-Trp-SPSB2

Construction of the 5-F-Trp-SPSB2 protein, and ^{19}F NMR determination of thiazole binding, were carried out as described previously.[28] ^{19}F NMR studies were carried out at 30 °C in 50 mM

Phosphate, 50 mM NaCl (pH 7.4), with a 5-F-Trp-SPSB2 concentration of 100 μM, with or without 500 μM control peptide or 3 mM **1**, as indicated.

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ABBREVIATIONS USED

2-AT, 2-aminothiazole; AMA1, apical membrane antigen 1; CAII, carbonic anhydrase II; CSP, chemical shift perturbation; FBDD, fragment-based drug discovery; HSQC, heteronuclear single quantum correlation; HTS, high-throughput screening; MIPS, Monash Institute of Pharmaceutical Sciences; NMR, nuclear magnetic resonance; PAINS, Pan Assay INterference compoundS; PrATs, Promiscuous 2-AminoThiazoles; SPSB2, SPRY domain-containing SOCS box protein 2; SAR, structure-activity relationships; STD, saturation transfer difference; WEHI, Walter and Eliza Hall Institute.

ASSOCIATED CONTENT

Supplementary Material

Supplementary material is available, which contains SPR sensorgrams of positive controls and selected compounds, STD, HSQC-NMR and SPR-derived binding data for the different targets, 2-AT PAINS subclasses and frequent hitter incidence. This material is free of charge via the Internet at http://pubs.acs.org.

REFERENCES

(1) Shuker, S. B.; Hajduk, P. J.; Meadows, R. P.; Fesik, S. W. Discovering High-Affinity Ligands for Proteins: SAR by NMR. *Science* **1996**, *274*, 1531–1534.

(2) Erlanson, D. A.; McDowell, R. S.; O'Brien, T. Fragment-Based Drug Discovery. *J. Med. Chem.* **2004**, *47*, 3463–3482.

(3) Tsai, J.; Lee, J. T.; Wang, W.; Zhang, J.; Cho, H.; Mamo, S.; Bremer, R.; Gillette, S.; Kong, J.; Haass, N. K.; Sproesser, K.; Li, L.; Smalley, K. S. M.; Fong, D.; Zhu, Y.-L.; Marimuthu, A.; Nguyen, H.; Lam, B.; Liu, J.; Cheung, I.; Rice, J.; Suzuki, Y.; Luu, C.; Settachatgul, C.; Shellooe, R.; Cantwell, J.; Kim, S.-H.; Schlessinger, J.; Zhang, K. Y. J.; West, B. L.; Ben Powell; Habets, G.; Zhang, C.; Ibrahim, P. N.; Hirth, P.; Artis, D. R.; Herlyn, M.; Bollag, G. Discovery of a Selective Inhibitor of Oncogenic B-Raf Kinase with Potent Antimelanoma Activity. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 3041–3046.

(4) LaPlante, S. R.; Padyana, A. K.; Abeywardane, A.; Bonneau, P.; Cartier, M.; Coulombe, R.; Jakalian, A.; Wildeson-Jones, J.; Li, X.; Liang, S.; McKercher, G.; White, P.; Zhang, Q.; Taylor, S. J. Integrated Strategies for Identifying Leads That Target the NS3 Helicase of the Hepatitis C Virus. *J. Med. Chem.* **2014**, *57*, 2074–2090.

(5) Edfeldt, F. N. B.; Folmer, R. H. A.; Breeze, A. L. Fragment Screening to Predict Druggability (Ligandability) and Lead Discovery Success. *Drug Discov. Today* **2011**, *16*, 284–287.

(6) Fink, T.; Bruggesser, H.; Reymond, J.-L. Virtual Exploration of the Small-Molecule Chemical Universe Below 160 Daltons. *Angew. Chem. Int. Ed. Engl.* **2005**, *44*, 1504–1508.

(7) Congreve, M.; Chessari, G.; Tisi, D.; Woodhead, A. J. Recent Developments in Fragment-Based Drug Discovery. *J. Med. Chem.* **2008**, *51*, 3661–3680.

(8) 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.

(9) Murray, C. W.; Carr, M. G.; Callaghan, O.; Chessari, G.; Congreve, M.; Cowan, S.; Coyle, J. E.; Downham, R.; Figueroa, E.; Frederickson, M.; Graham, B.; McMenamin, R.; O'Brien, M. A.; Patel, S.; Phillips, T. R.; Williams, G.; Woodhead, A. J.; Woolford, A. J.-A. Fragment-Based Drug Discovery Applied to Hsp90. Discovery of Two Lead Series with High Ligand Efficiency. *J. Med. Chem.* **2010**, *53*, 5942–5955.

(10) Bollag, G.; Hirth, P.; Tsai, J.; Zhang, J.; Ibrahim, P. N.; Cho, H.; Spevak, W.; Zhang, C.; Zhang, Y.; Habets, G.; Burton, E. A.; Wong, B.; Tsang, G.; West, B. L.; Powell, B.; Shellooe, R.; Marimuthu, A.; Nguyen, H.; Zhang, K. Y. J.; Artis, D. R.; Schlessinger, J.; Su, F.; Higgins, B.; Iyer, R.; D'Andrea, K.; Koehler, A.; Stumm, M.; Lin, P. S.; Lee, R. J.; Grippo, J.; Puzanov, I.; Kim, K. B.; Ribas, A.; McArthur, G. A.; Sosman, J. A.; Chapman, P. B.; Flaherty, K. T.; Xu, X.; Nathanson, K. L.; Nolop, K. Clinical Efficacy of a RAF Inhibitor Needs Broad Target Blockade in BRAF-Mutant Melanoma. *Nature* **2010**, *467*, 596–599.

(11) Bollag, G.; Tsai, J.; Zhang, J.; Zhang, C.; Ibrahim, P.; Nolop, K.; Hirth, P. Vemurafenib: the First Drug Approved for BRAF-Mutant Cancer. *Nat. Rev. Drug Discov.* **2012**, *11*, 873–886.

(12) Doak, B. C.; Morton, C. J.; Simpson, J. S.; Scanlon, M. J. Design and Evaluation of the Performance of an NMR Screening Fragment Library. *Aust. J. Chem.* **2014**, *66*, 1465–1472.

(13) Baurin, N.; Aboul-Ela, F.; Barril, X.; Ben Davis; Drysdale, M.; Dymock, B.; Finch, H.; Fromont, C.; Richardson, C.; Simmonite, H.; Hubbard, R. E. 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.

(14) Baell, J. B.; Holloway, G. A. New Substructure Filters for Removal of Pan Assay Interference Compounds (PAINS) From Screening Libraries and for Their Exclusion in Bioassays. *J. Med. Chem.* **2010**, *53*, 2719–2740.

(15) Hansch, C.; Fujita, T. P-Σ-Π Analysis. a Method for the Correlation of Biological Activity and Chemical Structure. *J. Am. Chem. Soc.* **1964**, *86*, 1616–1626.

(16) Baell, J. B.; Ferrins, L.; Falk, H.; Nikolakopoulos, G. PAINS: Relevance to Tool Compound Discovery and Fragment-Based Screening. *Aust. J. Chem.* **2013**, *66*, 1483–1494.

(17) Camps, M.; Rückle, T.; Ji, H.; Ardissone, V.; Rintelen, F.; Shaw, J.; Ferrandi, C.; Chabert, C.; Gillieron, C.; Françon, B.; Martin, T.; Gretener, D.; Perrin, D.; Leroy, D.; Vitte, P.-A.; Hirsch, E.; Wymann, M. P.; Cirillo, R.; Schwarz, M. K.; Rommel, C. Blockade of PI3Kgamma Suppresses Joint Inflammation and Damage in Mouse Models of Rheumatoid Arthritis. *Nat. Med.* **2005**, *11*, 936–943.

(18) Huth, J. R.; Mendoza, R.; Olejniczak, E. T.; Johnson, R. W.; Cothron, D. A.; Liu, Y.; Lerner, C. G.; Chen, J.; Hajduk, P. J. ALARM NMR: a Rapid and Robust Experimental Method to Detect Reactive False Positives in Biochemical Screens. *J. Am. Chem. Soc.* **2005**, *127*, 217–224.

(19) Huth, J. R.; Song, D.; Mendoza, R. R.; Black-Schaefer, C. L.; Mack, J. C.; Dorwin, S. A.; Ladror, U. S.; Severin, J. M.; Walter, K. A.; Bartley, D. M.; Hajduk, P. J. Toxicological Evaluation of Thiol-Reactive Compounds Identified Using a La Assay to Detect Reactive Molecules by Nuclear Magnetic Resonance. *Chem. Res. Toxicol.* **2007**, *20*, 1752–1759.

(20) Metz, J. T.; Huth, J. R.; Hajduk, P. J. Enhancement of Chemical Rules for Predicting Compound Reactivity Towards Protein Thiol Groups. *J. Comput. Aided Mol. Des.* **2007**, *21*, 139–144.

(21) Mjambili, F.; Njoroge, M.; Naran, K.; De Kock, C.; Smith, P. J.; Mizrahi, V.; Warner, D.; Chibale, K. Synthesis and Biological Evaluation of 2-Aminothiazole Derivatives as Antimycobacterial and Antiplasmodial Agents. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 560–564.

(22) Lim, S. S.; Debono, C. O.; MacRaild, C. A.; Chandrashekaran, I. R.; Dolezal, O.; Anders,

R. F.; Simpson, J. S.; Scanlon, M. J.; Devine, S. M.; Scammells, P. J.; Norton, R. S. Development of Inhibitors of Plasmodium Falciparum Apical Membrane Antigen 1 Based on Fragment Screening. *Aust. J. Chem.* **2013**, *66*, 1530–1536.

(23) Chuprina, A.; Lukin, O.; Demoiseaux, R.; Buzko, A.; Shivanyuk, A. Drug- and Lead-Likeness, Target Class, and Molecular Diversity Analysis of 7.9 Million Commercially Available Organic Compounds Provided by 29 Suppliers. *J. Chem. Inf. Model.* **2010**, *50*, 470–479.

(24) Mayer, M.; Meyer, B. Characterization of Ligand Binding by Saturation Transfer Difference NMR Spectroscopy. *Angew. Chem. Int. Ed. Engl.* **1999**, *38*, 1784–1788.

(25) Kuang, Z.; Lewis, R. S.; Curtis, J. M.; Zhan, Y.; Saunders, B. M.; Babon, J. J.; Kolesnik, T. B.; Low, A.; Masters, S. L.; Willson, T. A.; Kedzierski, L.; Yao, S.; Handman, E.; Norton, R. S.; Nicholson, S. E. The SPRY Domain-Containing SOCS Box Protein SPSB2 Targets iNOS for Proteasomal Degradation. *J. Cell Biol.* **2010**, *190*, 129–141.

(26) Hopkins, A. L.; Groom, C. R.; Alex, A. Ligand Efficiency: a Useful Metric for Lead Selection. *Drug Discov. Today* **2004**, *9*, 430–431.

(27) Supuran, C. T.; Scozzafava, A. Carbonic Anhydrase Inhibitors and Their Therapeutic Potential. *Exp. Opin. Ther. Patents* **2000**, *10*, 575–600.

(28) Leung, E. W. W.; Yagi, H.; Harjani, J. R.; Mulcair, M. D.; Scanlon, M. J.; Baell, J. B.; Norton, R. S. (19) F NMR as a Probe of Ligand Interactions with the iNOS Binding Site of SPRY Domain-Containing SOCS Box Protein 2. *Chem. Biol. Drug Des.* **2014**, *84*, 616–625.

(29) Baell, J. B. Broad Coverage of Commercially Available Lead-Like Screening Space with Fewer Than 350,000 Compounds. *J. Chem. Inf. Model.* **2013**, *53*, 39–55.

(30) M Nissink, J. W.; Blackburn, S. Quantification of Frequent-Hitter Behavior Based on Historical High-Throughput Screening Data. *Future Med. Chem.* **2014**, *6*, 1113–1126.

(31) Bruneau, P. Search for Predictive Generic Model of Aqueous Solubility Using Bayesian

Neural Nets. *J. Chem. Inf. Model.* **2001**, *41*, 1605–1616.

(32) Bruneau, P.; McElroy, N. R. logD7.4 Modeling Using Bayesian Regularized Neural Networks. Assessment and Correction of the Errors of Prediction. *J. Chem. Inf. Model.* **2006**, *46*, 1379–1387.

(33) Tarcsay, Á.; Keserü, G. M. Contributions of Molecular Properties to Drug Promiscuity. *J. Med. Chem.* **2013**, *56*, 1789–1795.

(34) Lessene, G.; Czabotar, P. E.; Sleebs, B. E.; Zobel, K.; Lowes, K. N.; Adams, J. M.; Baell, J. B.; Colman, P. M.; Deshayes, K.; Fairbrother, W. J.; Flygare, J. A.; Gibbons, P.; Kersten, W. J. A.; Kulasegaram, S.; Moss, R. M.; Parisot, J. P.; Smith, B. J.; Street, I. P.; Yang, H.; Huang, D. C. S.; Watson, K. G. Structure-Guided Design of a Selective BCL-X(L) Inhibitor. *Nat. Chem. Biol.* **2013**, *9*, 390–397.

(35) Nissink, J. W. M.; Schmitt, S.; Blackburn, S.; Peters, S. Stratified High-Throughput Screening Sets Enable Flexible Screening Strategies From a Single Plated Collection. *J. Biomol. Screen.* **2014**, *19*, 369–378.

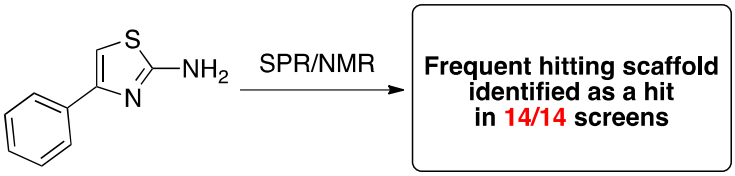
(36) Hodder, A. N.; Crewther, P. E.; Anders, R. F. Specificity of the Protective Antibody Response to Apical Membrane Antigen 1. *Infect. Immun.* **2001**, *69*, 3286–3294.

(37) Rich, R. L.; Errey, J.; Marshall, F.; Myszka, D. G. Biacore Analysis with Stabilized G-Protein-Coupled Receptors. *Anal. Biochem.* **2011**, *409*, 267–272.

(38) Giannetti, A. M. From Experimental Design to Validated Hits a Comprehensive Walk-Through of Fragment Lead Identification Using Surface Plasmon Resonance. *Meth. Enzymol.* **2011**, *493*, 169–218.

(39) Harris, K. S.; Casey, J. L.; Coley, A. M.; Masciantonio, R.; Sabo, J. K.; Keizer, D. W.; Lee, E. F.; McMahon, A.; Norton, R. S.; Anders, R. F.; Foley, M. Binding Hot Spot for Invasion Inhibitory Molecules on Plasmodium Falciparum Apical Membrane Antigen 1. *Infect. Immun.* **2005**, *73*, 6981–6989.

Table of Contents Graphic



Promiscuous 2-AminoThiazoles