Fragment Based Strategies for Discovery of Novel HIV-1 Reverse Transcriptase and Integrase Inhibitors

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Abstract: Human immunodeficiency virus (HIV) remains a global health problem. While combined antiretroviral therapy has been successful in controlling the virus in patients, HIV can develop resistance to drugs used for treatment, rendering available drugs less effective and limiting treatment options. Initiatives to find novel drugs for HIV treatment are ongoing, although traditional drug design approaches often focus on known binding sites for inhibition of established drug targets such as reverse transcriptase and integrase. These approaches tend towards generating more inhibitors in the same drug classes already used in the clinic. Lack of diversity in antiretroviral drug classes can result in limited treatment options, as cross-resistance can emerge to a whole drug class in patients treated with only one drug from that class. A fresh approach in the search for new HIV-1 drugs is fragment-based drug discovery (FBDD), a validated strategy for drug discovery based on using smaller libraries of low molecular weight (<300 Da) screened using primarily biophysical assays. FBDD is aimed at not only finding novel drug molecules but also probing the target protein to find new, often allosteric, inhibitory binding sites. Several fragment-based strategies have been successful in identifying novel inhibitory sites or scaffolds for two proven drug targets for HIV-1, reverse transcriptase and integrase. While any FBDD-generated HIV-1 drugs have yet to enter the clinic, recent FBDD initiatives against these two well-characterised HIV-1 targets have reinvigorated antiretroviral drug discovery and the search for novel classes of HIV-1 drugs.

Keywords: antiretrovirals, HIV-1, fragment-based drug discovery, reverse transcriptase, integrase, fragment screening.

1. HUMAN IMMUNODEFIENCY VIRUS (HIV) REMAINS A GLOBAL HEALTH ISSUE

Human immunodeficiency virus (HIV) was identified as the causative agent of acquired immunodeficiency syndrome (AIDS) over 30 years ago [1-4]. HIV is a retrovirus that infects CD4+ immune cells and is primarily sexually transmitted, although infections also occur by transfer of infected blood such as needlestick injuries or needle-sharing among injecting drug users. If an HIV infection is left untreated, the eventual depletion of the CD4+ T lymphocytes leads to severe and fatal immunodeficiency. HIV remains a major burden on health resources worldwide as approximately 35 million people are infected [5] and there is currently neither a cure for HIV infection nor an effective vaccine to block HIV transmission. There are, however, over 25 antiretroviral drugs (ARVs) used to treat HIV. ARVs are usually administered as a cocktail of two or more drugs, a regime that was initially termed highly active antiretroviral therapy (HAART), but now referred to as combined antiretroviral therapy (cART).

Treatment with cART can control viral loads and prevent development of AIDS, although it cannot eradicate the virus completely so HIV positive individuals must maintain continuous drug therapy throughout their lives. Viral suppression by cART has been highly successful for decreasing morbidity and mortality associated with HIV infection [6-11], providing patients with a good quality of life in the absence of a permanent cure.

The effectiveness of cART is often limited by the emergence of drug-resistance mutations and the availability of resources for monitoring virological failure due to these mutations (reviewed in [12]). Like many other viral and bacterial pathogens, HIV can rapidly develop resistance mutations under the selective pressure of drug treatment and those that favour survival of the virus persist in the genome. Mutations constantly emerge in the HIV genome due to the high replication rate, the high error rate and recombination mediated by reverse transcriptase [13], an HIV enzyme that generates a double-stranded DNA copy of the viral RNA genome. Without monitoring of patients on cART, their viral loads can increase and drug resistance mutations can emerge. These mutations can circumvent their current combination of ARVs and therefore severely limit the number of drugs available for future treatment and/or result in transmission of drug-resistant HIV strains [14]. Recent surveillance has shown that transmission of drug-resistant strains is on the
The continual development of drug resistant HIV strains, influence of associated side effects of some ARVs and problems with adherence and failure of cART in treatment-naïve and treatment-experienced patients drives a continuous need for new classes of antiretrovirals. To address these problems, there are currently several ARV drugs in clinical development (Table 1). Some are new drugs that fall into known classes and others are new classes of ARV drugs, targeting other proteins in the viral life-cycle. While the identification of new HIV drug targets is a promising approach for generating diverse drug classes, many programs remain focussed on the proven targets RT and IN. Here, we highlight recent studies for defining novel drug scaffolds using a newer approach called FBDD for development of novel antiretrovirals against HIV-1 RT and IN.

3. USING FRAGMENT BASED DRUG DISCOVERY

3.1. What are “fragments” and why use FBDD?

Current industrial drug discovery programs are initiated using high-throughput screening (HTS) of large chemical libraries consisting of $10^5$-$10^6$ compounds in a search for detectable activity in a biological assay. The compound “hits” identified in such a screen would typically have activities in the low micro- to nanomolar range deriving from a level of intrinsic chemical specificity for their target. Conversely, FBDD, also referred to as fragment based ligand design or fragment based lead generation, is a relatively new approach designed to identify minimally functionalised compounds as chemical building blocks for strategic optimisation into larger potent drug molecules [44]. The core principle of FBDD is that small compounds (fragments) are capable of making high quality interactions with the target protein and, even though their affinity for the protein is low, they can be elaborated into larger, high affinity inhibitors with good biopharmaceutical properties [45]. FBDD is also distinct in its focus on biophysical methods to assess binding affinity rather than exclusively focussed on biological
activity assays to prioritise chemical compounds. FBDD screening requires far fewer compounds to yield hits than conventional library screening as the fragments are smaller (MW <300) and are able to cover a greater amount of chemical space and diversity as they represent less complex starting scaffolds [46]. With a predicted $10^3$ possible molecules with 30 heavy atoms or less [47], simplifying the starting scaffolds to fragment sized compounds (<300 MW) means fewer molecules need to be screened to achieve similar coverage of chemical diversity as a collection of hundreds of thousands of larger, more functionalised compounds (>300 MW). The FBDD ethos is that fragments are far better suited as starting scaffolds for innovative molecular design and chemical elaboration in order to produce better quality leads than traditional drug design strategies [44, 48].

Shuker et al [49] and Hajduk et al [50] reported the first practical fragment-based screening approaches in the mid 1990s called ‘SAR by NMR’ - structure-activity relationship (SAR) by nuclear magnetic resonance (NMR). Since then, FBDD has been used to generate drug candidates for many drug targets and has been validated by the FDA’s approval of vemurafenib (Zelboraf, PLX4032) in 2011, Plexxikon’s drug for treatment of metastatic melanoma [51-53]. Several other compounds generated using FBDD are currently under evaluation in clinical trials including three in Phase III studies for various indications including Alzheimer’s disease (MK-8931, BACE1 inhibitor) [54-55], breast cancer (LEE011, CDK4 inhibitor) [56] and lymphocytic leukemia (ABT-199, Bcl-2 inhibitor) as well as almost 30 more candidates in Phase I and II trials (www.clinicaltrials.gov).

3.2. Fragment-based approaches are driven by binding efficiency

The small size of fragment hits means they have low affinity for their target (usually high μM to mM). However, low affinity does not necessarily mean that the functional groups within the molecule are not individually making strong interactions with the target protein, just that there are few such interactions. Consequently, FBDD programs have adopted efficiency metrics that establish binding quality, by combining measures of compound affinity (or potency) with other optimisable parameters such as molecular size or lipophilicity. One of the most widely used metrics is ligand efficiency (LE) which the free energy of binding ($\Delta G_{\text{bind}} = -RT \ln K_d$) and the number of non-hydrogen or heavy atoms to assess the contribution of each atom in the compound to the overall binding interaction with its protein target [57-58]:

$$LE = \frac{\Delta G_{\text{bind}}}{\text{HAC}} = -RT \ln \left( K_d \right) / \text{HAC}$$

where LE is ligand efficiency, R is the ideal gas constant, T is temperature in Kelvin, $K_d$ is the dissociation constant for the binding interaction between the compound and the target protein and HAC is the “heavy atom count” or number of non-hydrogen atoms in the compound. While fragments have low affinity, they have only a small number of non-hydrogen atoms indicating that most of the molecule is contributing to binding. Constraints on the ultimate size of the target compound (e.g. MW 500) and potency (e.g. 10 nM) dictate a minimum LE, which is often considered to be 0.3 kcal/mol/HAC although target LE values may vary [59]. Other efficiency metrics can be used to evaluate ligand quality; IC$_{50}$ divided by MW provides the binding efficiency index (BEI) [60] which is appropriate if only potency data is available. Other optimisation parameters can also be included in the calculation such as the partition coefficient, logP (ratio of concentration of the compound in octanol to its concentration in water), an indication of compound hydrophobicity, to generate ligand lipophilicity index (LLE$_{AT}$) [61], or even polar surface area for the surface efficiency index (SEI) [62]. This list is by no means exhaustive. While LE and other indices relating binding affinity to molecular size/shape have been adopted widely for FBDD and HTS programs, their value for decision making in drug discovery has recently been debated in the literature [63-65].

One of the key concepts underlying FBDD is that the careful selection of low MW and low lipophilicity (logP) compounds during library design, screening and hit validation can provide a greater chance of producing such favourable properties in the late stages of generating drug candidates [48]. Lipinski [66] famously proposed the ‘rule of five’, defining favourable physical properties for drug-like compounds based on the properties of oral drugs that had reached the market. The success of FBDD in preserving favourable properties is difficult to evaluate given the diversity in strategy and priorities in any given drug discovery program; however, two separate analyses of available FBDD data, Astex [48] and Astra-Zeneca [67] sought to evaluate whether fragments generate leads with more favourable properties than larger, more conventional HTS library hits. Both studies are in agreement that fragment hits and their corresponding lead compounds are significantly smaller and less lipophilic when compared to a set of published HTS drugs.

3.3. The FBDD pipeline and methodology

FBDD approaches can be initiated on a much smaller scale than conventional drug screening which otherwise limits drug screening programs to the better resourced laboratories with access to large compound collections typically found in the pharmaceutical industry. Consequently, FBDD has been very popular for initiating drug discovery programs in academic settings that are intellectually invested in identifying and characterising innovative drug targets and are already suitably equipped with sensitive instrumentation for detecting weakly binding molecules. Smaller libraries of fragment-sized compounds are accessible to both academic and industry-based laboratories and can be purchased from commercial sources, or compiled in-house based on library design principles derived from over a decade of FBDD programs (reviewed in [68-69]).

The FBDD toolkit often consists of multiple complementary biophysical and structural biology methods used in combination to screen a fragment library against a desired target [46]. NMR [70] and surface plasmon resonance (SPR) [71-72] are popular first-pass screening methods as they are rapid and very sensitive. Ligand-observed NMR methods (such as saturation transfer difference NMR or STD-NMR) are advantageous as the size of the protein target is not a limiting factor. With hardware and software now available for high throughput crystal screening, data measurement and processing, X-ray crystallography can be extremely informative in the screening phase for suitable targets [54, 73-75] as it can
detect binding as well as elucidate the structural details of the fragment binding site in one step. Both NMR and crystallography can resolve signals from cocktails of compounds so libraries can be screened in mixtures of 5-10 compounds to decrease sample number and screening time [76]. Different methods used for fragment screening have also been reported to produce distinct sets of hits when using the same fragment library against the same protein target [77]. Using clever experimental design and high-throughput advancements, other biophysical methods have more recently been optimised for use in fragment screening and validation such as mass spectrometry [78-79], isothermal titration calorimetry [80-82] and weak affinity chromatography [83-86].

Once hits have been confirmed to inhibit or bind to the target, a medicinal chemistry program can be established to elaborate the fragment scaffold into a suitable lead candidate. During this process, ligand affinities are measured using biophysical methods and/or bioassays and are almost always guided by X-ray structures of the fragment hits bound to the protein target which help to characterise binding sites and guide the medicinal chemistry. In most cases, fragment elaboration proceeds by fragment ‘growing’ but in some cases, multiple scaffolds can be ‘merged’ or linked to rapidly generate high affinity compounds.

4. FBDD AND HIV-1 REVERSE TRANSCRIPTASE

4.1. HIV-1 RT structure and known drug binding sites

HIV-1 RT is an essential viral enzyme that converts the single-stranded viral RNA genome to a double-stranded DNA copy to be integrated into the host genomic DNA. RT acts early in the viral life cycle, soon after entry to the host cell mediated by the HIV-1 envelope complex. It is a multi-functional enzyme with both RNA-dependent and DNA-dependent DNA polymerase activities as well as RNase H activity, which is responsible for digesting RNA in the RNA-DNA duplex. All three functions are necessary to generate a double-stranded DNA copy of the viral RNA genome [21, 87]. HIV-RT is a heterodimer comprised of the p66 and p51 subunits, where p66 is catalytic subunit and p51 is structural. It has been described as a hand-shaped molecule where p66 forms the polymerase domain from fingers, thumb and palm subdomains, as well as a connection subdomain that joins the RNase H and polymerase domains [88-89]. The nucleic acid substrate, or template/primer (T/P), binds in a groove that runs through both the polymerase and RNase H active sites. Only two classes of HIV RT inhibitor are available for clinical use, the NRTI and NNRTIs, which have been described in section 2 above.

There are four NNRTIs in clinical use, two first generation NNRTIs, nevirapine and efavirenz, and two next-generation NNRTIs, rilpivirine and etravirine [90]. The NNRTIs show more variety in their chemical structures compared to the NRTIs, although all four inhibit enzyme function by binding the allosteric NNIBP. While cross-resistant HIV-1 mutants have been reported for the clinically-available NNRTIs extensive structural analyses suggest that properties such as strategic inhibitor flexibility could help maintain a high genetic barrier to clinically-relevant resistance mutations that arise in the NNIBP [91-93].

Other classes of RT inhibitors have been reported that are under development but have not yet reached the clinic (Table 2). Nucleotide-competing RT inhibitors (NCRTIs) are distinct from NRTIs as they are not nucleoside analogs and do not cause chain termination. No inhibitors of HIV-1 RT RNase H activity are in clinical use although there have been numerous studies in the area (Table 2). Inhibitors of HIV-1 RT dimerisation, T/P binding and inhibitors that bind to multiple sites are also subject of recent investigations (Table 2). Maximising the number of lead ARV candidates entering clinical evaluation is essential to increase the possibility that drugs in novel classes will be available for clinical use given the high failure rate in progressing a drug lead through the preclinical and clinical pathway to approval. A greater number of drug classes available for use in cART could drastically reduce the ability of HIV to develop viable drug-resistant strains, and thus increase the efficacy of cART during long-term use.

4.2. HIV-1 RT as a target for FBDD strategies

HIV-1 RT is a highly flexible enzyme [94]. This flexibility makes it an ideal target for allosteric inhibition, particularly since it is known that inhibitors bound to one site can affect the enzyme at a distant location. For example, NNRTIs can affect HIV-1 RT RNase H activity and enhance dimerisation as well as primarily blocking polymerase activity (Section 2). A wealth of structural information is available for this target, with over 200 crystal structures of HIV-1 RT in the Protein Data Bank (PDB) (http://www.rcsb.org/), in apo form or bound to various inhibitors and/or substrate variants. One advantage of an FBDD approach is that fragments are small enough to act as probes to identify allosteric binding sites of a conformationally flexible target. Depending on the screening strategy used, fragment screens have the potential to either find novel chemical scaffolds that target clinically relevant binding sites, or to probe for new allosteric binding sites to exploit for drug design. Both approaches have been used in three FBDD programs against HIV-1 RT.

4.3. FBDD screening against HIV-1 RT to find novel NNRTI scaffolds

Gettmann and colleagues at Beactica AB (Sweden) [95] used an FBDD approach to identify novel scaffolds as a basis for developing new NNRTIs with activity against well-known drug-resistant variants of HIV-1. Their approach was to find scaffolds that likely bind to the NNIBP of wild-type HIV-1 RT using a combination of a binding assay and an activity assay. A library of ~1000 fragment sized compounds (MW <300 Da) was first screened for binding to HIV-1 RT using a SPR assay [96] yielding 96 fragments (~9% library). These hits were then simultaneously screened for inhibition of RT polymerase activity in an enzymatic assay as well as for their ability to compete with the NNRTI, nevirapine, for binding to the NNIBP. Only 8 compounds competed with nevirapine and reproducibly demonstrated an IC_{50} value less than 1 mM. The final screen was resistance profiling to find compounds that maintained activity against the drug-resistant RT variants (K103N, Y181C, L100I). In the final analysis, one of the 8 fragments, compound 1 (Table 3), had reproducible inhibitory activity in the enzymatic assay against all four variants of HIV-1 RT (IC_{50} < 25 µM).
FBDD offers several advantages for a drug discovery program aimed at finding novel NNRTI scaffolds. NNRTIs bind to HIV-1 RT by an “induced fit” mechanism, where the binding pocket, NNIBP, is evident only when an inhibitor is bound [21]. Due to their small molecular size compared to compounds used for conventional HTS, fragments could be better able to probe the NNIBP for new binding modes with the ability to evade resistance mutations as they arise. Conventional screening strategies are usually based on broad activity assays and use very large libraries (>10^10) of highly functionalised compounds that would be much less likely to have yielded similar numbers of hits. One novel scaffold from a library of ~1000 compounds represents a very respectable hit rate of 0.1%. The potency threshold for a desirable compound is also likely to be much more stringent for larger compounds in HTS, limiting the likelihood of identifying compounds as hits, and for progressing to competition or resistance screens so early in the process. Because Geitman et al [95] could incorporate selective screening strategies like the competition assay and resistance profiling so soon after the initial library screen, they were able to identify a novel NNRTI scaffold with desired inhibitory properties from a small number of compounds in only three screening steps.

The path required to chemically evolve a low potency/low affinity fragment scaffold with desirable properties into a high potency NNRTI drug lead is less clear. No further reports describing optimisation of 1 (Table 3) into a novel NNRTI lead or candidate have been published, although the authors of the SPR fragment screening study [95] published a second study in the same issue of J Med Chem [97] analysing the effectiveness of fragment-based strategies to find NNRTI fragment scaffolds binding to HIV-1 RT. The authors focused their analysis on understanding how best to optimise fragments into high potency leads by characterising fragment-sized compounds derived directly from substructures of known NNRTIs with nanomolar binding affinity constants (K_d). Surprisingly, they found that many of the NNRTI substructures had very weak binding affinities and low LE values compared to the parent NNRTI. Furthermore, analysis of binding energies of the NNIBP led to this group reporting that there might not be a fragment binding site or “hotspot” in the NNIBP. These findings highlight challenges faced in elaborating smaller, weaker binding fragments into higher potency compounds against a conformationally flexible target such as HIV-1 RT.

4.4. FBDD screening against HIV-1 RT to identify novel allosteric binding sites for drug design

The Arnold group at Rutgers University took an alternate approach and used fragment screening to search for novel allosteric binding sites rather than mining a known inhibitory pocket [98]. A library of 742 fragment-sized molecules was screened by X-ray crystallography, discovering 34 fragments that bind to RT at 16 different binding sites. Seven of these sites have been reported in detail [98]. By blocking the NNIBP during the screening process using rilpivirine, their screen was able to select for fragments that bound to previously unknown allosteric pockets. The seven identified binding sites are located in the polymerase domain (incoming nucleotide binding site, knuckles, NNRTI adjacent and 399) and the RNase H domain of HIV-1 RT (428, RNase H primer grip adjacent, 507).

Three of the seven novel binding sites were demonstrated to be inhibitory when the fragments bound in these pockets were tested in a dual activity assay that detects inhibition of either RNase H or DNA polymerase activity simultaneously. The fragments bound in these sites have varying potency in the mid-micromolar range (Table 3). Based on the structural analysis, these three binding sites have differing potential as druggable sites. The “NNRTI adjacent” binding site is located near the NNIBP binding site identified by the binding of 2 (Table 3). This site is located at the dimerization interface between the RT subunits. Compound 2 made contacts with side chains from both p66 and p51 (Figure 1A). Although this pocket is discrete and contains conserved residues less likely to tolerate mutation under selective drug pressure, the authors note that any inhibitors designed to bind to the NNRTI adjacent pocket would need to address less conserved residues forming key inhibitor interactions such as T165 and T139, and NNRTI-resistant mutations such as Y181C and E138K. The second novel inhibitor site described by Bauman and colleagues [98] was the Incoming Nucleotide Binding Site, located adjacent to both the polymerase active site and the nucleotide binding pockets of RT. Movement of residue Q151 on binding of 3 (Table 3) opens up the pocket, where compound 3 makes contacts with a catalytic aspartate (D185) as well as other highly conserved residues that comprise the pocket (Figure 1B). The authors highlight this pocket as one of the two most appealing for drug design initiatives against RT as an inhibitor bound here could directly interfere with both nucleotide binding and polymerase catalytic activity. The third novel inhibitory binding site that was identified as very appealing for future drug design is the “Knuckles” site, referring to its location between the p66 “fingers” and “palm” domains of the hand-shaped RT molecule. This site has been characterised by structures of RT bound to three separate compounds, 4, 5 and 6 (Table 3) that have defined the flexibility in this pocket and suggest several opportunities for elaborating fragments. Although only compound 5 was found to be inhibitory [98](Table 3), the trifluoromethoxy and phenol groups of compounds 4 and 5 make key interactions in the Knuckles pocket, exposing S117 and M164. Bauman et al suggest expansion at residue I167, as movement of K166 allows binding of larger compounds such as 6 (Figure 1C). All three of these sites demonstrate potential for further elaboration of fragments or in de novo structure-based drug design towards developing new classes of HIV-1 RT inhibitors.

Crystallography has proven to be a powerful and highly informative tool to screen fragment libraries, as it not only determines binding to the target, but also the location of the binding site and direction for chemical elaboration of fragments. It does, however, require significant target-specific optimisation including the ability to generate very high resolution structures of fragment complexes. The Arnold group had to engineer a construct of HIV-1 RT, RT52A, to produce crystals that diffracted well enough to resolve fragments bound to RT [99]. While this fragment screening strategy was successful in identifying three inhibitory sites, the remaining four of the seven sites described were not found to be inhibitory. While lack of inhibition discourages pursuit for drug development, these
poor pockets could require larger inhibitors to exert any inhibitory effect [98]. For example, structural analysis of 7 (Table 3) bound to the RNase H Primer Grip Adjacent site suggests it could be expanded into the RNase H active site guided by the crystal structure to generate an inhibitor. Since publication of this crystallographic fragment screen in 2013 [98], there have been no further reports of inhibitors exploiting these novel sites.

4.5. FBDD screening against HIV-1 RT to discover novel allosteric inhibitors

In the most recently reported FBDD approach targeting HIV-1 RT, the Tachedjian group in Melbourne, Australia [100] used STD-NMR to screen a library of 630 fragments against HIV-1 RT to search for allosteric inhibitors at sites other than the NNIBP. STD-NMR was followed by a secondary screen using a HIV-1 RT polymerase activity assay, where eight fragment hits were identified with micromolar potencies against the DNA polymerase function of wild-type (WT) HIV-1 RT. Only three of the hits, 8-10 (Table 3) maintained a similar potency against WT RT and three clinically relevant NNRTI-resistant RT mutants, K103N, Y181C and G190A. In contrast to Bauman et al [98], this group [100] then used biochemical assays to gather evidence about the mechanisms of action and possible binding sites on the enzyme for the top three fragment hits. Two of these compounds, 9 and 10, were shown to compete with the HIV-1 RT substrates, nucleotide and T/P, respectively, suggesting that these two compounds could be inhibiting the enzyme allosterically with fragment 9 likely acting at or near the polymerase active site, such as the NcRTI binding site [101] or another allosteric site. Fragment 10 was also shown to inhibit RNase H activity, although it is unclear whether inhibition of both polymerase and RNase H activities are the result of binding at one or more sites on RT. While both these inhibitory mechanisms have been described in the literature for structurally unrelated inhibitors (Table 2), their binding sites could be distinct. Fragment 10 was even shown to have activity against HIV-1 replication in cell culture (EC₅₀ = 20 μM), likely at the reverse transcription step as determined in a time of addition assay. These properties are remarkable for such a small molecule with few distinctive chemical features, particularly since other known inhibitors of T/P (i.e. SY-3E4 [102], KM-1 [103] and APEX57219 [104], Table 3) are large and undesirable as drug development candidates. A small structure-activity relationship analysis revealed three related compounds with 3-5 fold higher potency than 8-10, and one of these related compounds, 11 (Table 3), was shown to maintain T/P competition and activity against HIV-1 in cell culture, similar to its parent compound, 8.

This study represents a novel approach to FBDD for this HIV-1 target. While the focus for FBDD is often on biophysical assays and characterization, it can be difficult to judge the appropriate timing to assess the inhibitory potential of allosteric inhibitors or binding sites. In this study [100], activity assays were used early in the screening process while taking steps to minimise false positives due to compound aggregation at high concentrations. Mechanistic assays were also used to characterise and prioritise hits from the fragment screen for further development. The fact the Tachedjian group [100] were able to identify additional compounds related to the top three fragment hits that have improved potency from only a small structure-activity study suggests that these scaffolds are promising candidates for further optimization in an ongoing drug development program. This approach shows that mechanistic characterization could be an effective tool in an FBDD program, particularly given the uncertainty of obtaining cocrystal structures of the compound hits attracting the greatest interest.

5. FBDD AND HIV-1 INTEGRASE

5.1. HIV-1 IN structure and known drug binding sites

HIV-1 IN catalyses the insertion of the double-stranded DNA copy of the viral genome, generated by RT, into the host chromosomal DNA [105]. The viral genome then persists in the host genome whereby the host transcription/translation machinery propagates both viral proteins and RNA to generate new viral particles. IN-mediated integration is a two-step process consisting of firstly, excision of a dinucleotide from the viral DNA called the “3'-processing step”, followed by “strand transfer reaction” integrating viral DNA into the host genome. The functional IN unit is a tetramer consisting of two active sites, where each IN monomer has three domains with distinct functions – an N-terminal domain (NDT) HHCC-type zinc finger for protein multimerisation, a central core domain (CCD) for DNA substrate recognition and containing the catalytic “DDE motif”, and a C-terminal domain (CTD) for non-specific DNA binding, stabilising the IN-DNA complex during translocation.

The three IN inhibitors currently in clinical use are INSTIs that bind to the catalytic IN active site. Other classes of IN inhibitors have been reported, primarily allosteric inhibitors targeting various sites on the IN CCD domain (Table 2). The most prominent class of allosteric IN inhibitors are the ALLINIs (allosteric IN inhibitors) that target a site on HIV-1 IN at the dimer interface that interacts with a host cell factor, lens epithelial-derived growth factor (LEDGF), which is essential for integration [106-109]. LEDGF tethers the IN-DNA complex to the chromosome, and is also packaged into virions [110]. The ALLINIs, first coined as LEDGINs (LEDGF-IN inhibitors) by the Debyser group [111], bind at the CCD-CCD interface of two IN subunits and compete with LEDGF for binding to the same pocket. These inhibitors have been the subject of extensive characterisation with some compounds in advanced preclinical development (reviewed in [112-114], Table 2). While there is not yet a clinical study evaluating the genetic barrier of ALLINIs to the emergence of HIV resistance mutations, in vitro selection has suggested that single mutations could confer drug resistance to this class although there is limited cross-resistance with the existing INSTIs [111, 115]. Although this class of inhibitors were designed to inhibit IN function to block HIV replication, ALLINIs also stabilise interacting IN subunits, and promote aberrant, higher order IN multimerization leading to impaired maturation of virus particles [115-116]. The effect on multimerization of IN is now being exploited directly as a strategy for drug development (Table 2)[117]. Other classes of inhibitors have been reported in a handful of studies including peptide-based inhibitors of the LEDGF interaction and multimerisation, as well as one inhibitor in a new class called allosteric, non-catalytic site inhibitors (NCINIs)(Table 2). Very few studies have been published on NCINIs, most likely due to the
proprietary nature of the lead compound [118-119], which has recently entered Phase I clinical evaluation (Table 1).

5.2. HIV-1 IN as a target for FBDD strategies

HIV-1 IN is an essential viral enzyme that has been a successful drug target for HIV-1 treatment, with three ARVs in clinical use – raltegravir, elvitegravir and dolutegravir. IN inhibitors are well tolerated in patients and the two newer IN inhibitors, elvitegravir and dolutegravir, have a high genetic barrier to resistance, which makes IN an attractive target for development of new ARVs. Nevertheless, HIV strains that are cross-resistant to IN inhibitors have already begun to emerge (section 2 above) as these inhibitors are all members of the same class, the IN strand transfer inhibitors (INSTIs). There is now increasing motivation to develop novel classes of IN inhibitors for HIV-1 treatment and prevention.

Like HIV-1 RT, HIV-1 IN is a multimeric flexible enzyme with the potential for novel allosteric sites perhaps with an “induced fit” mechanism that could be identified using fragment-sized probes in an FBDD screening program. IN is, however, a large tetrameric protein that has posed a greater structural challenge than RT. Currently no crystal structures are available for the full-length HIV-1 IN, although structures of the three isolated domains are available [120-123] and the crystal structure of a related retroviral integrase complex from prototype foamy virus [124] has been used to generate full-length homology models [125-126] that can be used for rational drug design against HIV-1 IN. Most drug discovery programs are using relevant portions of IN depending on the site to be targeted, as well as substantial engineering to introduce mutations to improve protein solubility for screening assays and structural studies [127-130] (see section 4.2 & 4.3).

Similar to the approaches used for HIV-1 RT, FBDD studies targeting HIV-1 IN have exploited both the LEDGF/IN interaction as well as searching for novel allosteric binding sites using a variety of fragment-based methodologies. In addition to a few computationally-based screens of virtual libraries [131-133], there have been two fragment-based screening studies targeting HIV-1 IN [128, 130] leading to several novel inhibitor scaffolds targeted to the LEDGF site and two new allosteric sites on HIV-1 IN, the “FBP” and “Y3 site”. These two screening programs are described below.

5.3. STD-NMR and SPR screening of a fragment library against HIV-1 IN

Two groups in Melbourne, Australia screened the same Maybridge fragment library against HIV-1 IN using two different approaches [77]. A group at Monash University used STD-NMR as the primary screen [128] while a second group at CSIRO used SPR [130] to screen the library. Both groups used X-ray crystallography to characterise fragment hits using a similar IN-CCD construct. Despite using almost the same set of compounds to screen against the same target, these two different approaches produced distinct sets of hits.

In the first approach, NMR screening of the Maybridge Ro3 fragment library of 500 compounds yielded 62 confirmed fragment hits [128]. Compounds were first screened as cocktails of ~5 compounds by STD-NMR then followed by $^{15}$N-HSQC of individual hits. Cocrystal structures of 15 of these fragment hits with HIV-1 IN$_{\text{CORE123}}$ (IN-CCD with four solubilising mutations C56S, W131D, W139D and F185H) were determined, revealing several fragments bound to a novel binding site, termed the fragment binding pocket (FBP), which is located at the dimer interface, adjacent to the LEDGF binding site. Two fragments bound in overlapping sites in the FBP, KM00835 (12) and SB00942 (13) (Table 4). Based on the position of the overlapping chemical groups, the thiophene of 12 and the aromatic ring of 13, the two compounds were merged to generate a series of four new compounds. Crystal structures of the new series bound to HIV-1 IN$_{\text{CORE123}}$ revealed that some features of the original compounds were maintained and bound with similar weak affinity (K_D ~ 3-6 mM). Introduction of a bromine to one of the compounds in the merged series, 14 (Table 4), displaced a key water-mediated hydrogen bond with the S195 side chain seen in the structure, IN$_{\text{CORE123-12}}$ (Figure 2A, left), and formed a direct interaction with S195 (Figure 2A, right). This new interaction was exclusive to one compound, 14, and resulted in a 17- to 35-fold improvement in the binding affinity [128] over the parent compounds (Table 4). The fragments and merged compound series were not tested in any integrase activity assays for their inhibitory potency and the functional relevance of the FBP remains unknown.

The second group screened the Maybridge Ro3 fragment library against HIV-1 IN using a combination of SPR and STD-NMR [130]. The library was purchased from Maybridge at slightly different time from Wielens et al [128] meaning that the two libraries had ~90% compounds in common. Crystal structures were determined for six of sixteen fragment hits from the screening phase, in complex with another optimised IN-CCD construct, IN$_{\text{CORE31}}$ [134]. One of these hits, a N-benzyl indolinone, 15 (Table 4) was bound in a novel pocket on HIV-1 IN defined by Q62, H183 and S147, located near the mobile loop at the dimer interface (Figure 2B). Compound 15 inhibited HIV-1 IN with micromolar potency (IC$_{50}$ = 259 µM) in a strand transfer assay. A similar binding site had been previously observed in a crystal structure of a related integrase from avian sarcoma virus (ASV) complexed with inhibitor Y3 [135]. Using X-ray crystallography, molecular modelling and the strand transfer assays, SAR studies of 19 compounds in three related chemical series, N-benzyl indoline-2,3-diones, N-benzyl indolin-2-ones, N-benzyl indoliones revealed several avenues for further development of potent inhibitors at this novel site. One compound, 16 (Table 4), was 45-fold more potent than the parent compound, 15. Molecular docking of compound 16 bound to HIV-1 IN-CCD suggests that the increase in potency potentially arises from strengthening interactions with residues Q62 and H183 that define the binding site. However, molecular dynamics simulations suggest that the conformation of HIV-1 IN that forms these key interactions with 16 is not the most energetically favourable and that this inhibitor could be binding in multiple conformations [136].

Two structurally similar fragments, 17 and 18 (Table 4), identified by Rhodes et al [130] were bound to the LEDGF site on HIV-1 IN, and their SAR was explored in a follow-up study [136]. Analogues of 17 and 18 were investigated using X-ray crystallography, a strand-transfer assay, a cell-based assay and the Alphascreen assay for the IN-LEDGF binding site [137] designed to specifically assess
compounds that compete for LEDGF binding to HIV-1 IN. The most promising compound from this study, 19 was active in both biochemical assays in the micromolar range as well as against HIV-1 replication in cell culture (EC_{50} = 29 μM).

The crystal structures of the IN-fragment complexes determined by Wielens et al [77, 128] have been used in subsequent virtual fragment screens against HIV-1 IN [133, 138] although no further development of the inhibitor scaffolds or exploitation of the novel binding sites from either study has been published to date. Since Peat et al [136] published the study describing the novel LEDGF inhibitors, the additional abilities of LEDGF inhibitors to promote aberrant HIV-1 IN multimerisation and HIV virion maturation have been characterised (see section 5.1.), suggesting that development of this class of inhibitors is not as straightforward as first anticipated. However, recent work [113, 116-117, 139] has provided a greater understanding of how these effects could be exploited in the development of novel classes of ARVs targeting HIV-1 IN.

CONCLUSION

There is a constant need for new ARVs to use for HIV-1 treatment and prevention strategies to combat the emergence of drug resistance. While ongoing research has identified new inhibitory binding sites on proven targets such as HIV-1 RT and HIV-1 IN, many of the ARVs currently in clinical trials for HIV-1 fall into the same classes as existing drugs in clinical use. While only a relatively new technique, FBDD has been successfully applied to identify high quality drug scaffolds and new inhibitory binding sites against HIV-1 RT and IN as discussed above, as well as several other HIV-1 drug targets of HIV-1 including PR, TAT and envelope proteins, gp120 and gp41. Whether fragments have a high genetic barrier to the development of drug resistance and if they are able to inhibit HIV that are resistant to existing drugs remains to be determined. Overall, FBDD-based programs are making a valuable contribution to the ongoing development of efficacious agents to be used in therapeutics and PrEP for HIV-1 worldwide.

CONFLICT OF INTEREST

The authors declare no conflict of interest. This work was supported by the National Health and Medical Research Council of Australia (NHMRC) (Project Grant 1064900) and the Percy Baxter Charitable Trust, Perpetual Trustees, awarded to C.F.L. and G.T. G. T. was supported by the NHMRC Senior Research Fellowship 543105.

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Table 1. New antiretroviral drugs in clinical trials.

<table>
<thead>
<tr>
<th>Drug Class</th>
<th>Target</th>
<th>Drug Name</th>
<th>Alternate Name/s</th>
<th>Company</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phase III</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NNRTI</td>
<td>HIV-1 RT</td>
<td>Dapivirine$^b$</td>
<td>TMC-120</td>
<td>Tibotec Pharmaceuticals/Janssen; Johnson &amp; Johnson</td>
<td>[140]</td>
</tr>
<tr>
<td>NNRTI</td>
<td>HIV-1 RT</td>
<td>Doravirine</td>
<td>MK-1439</td>
<td>Merck &amp; Co</td>
<td>[141]</td>
</tr>
<tr>
<td>NRTI</td>
<td>HIV-1 RT</td>
<td>Apricitabine$^b$</td>
<td>AVX-754; BCH-10618; BCH-10619; BCH-10652; SPD-754</td>
<td>Avexa</td>
<td>[142-143]</td>
</tr>
<tr>
<td>Entry</td>
<td>HIV-1 gp120</td>
<td>Fostemsavir</td>
<td>BMS-663068; BMS-626529</td>
<td>Bristol-Myers Squibb</td>
<td>[144]</td>
</tr>
<tr>
<td>Entry</td>
<td>CD4 receptor</td>
<td>Ibalizumab$^c$</td>
<td>TNX-355; Hu5A8</td>
<td>Tanox; Biogen Idec; Taimed Biologies</td>
<td>[145]</td>
</tr>
<tr>
<td>Fusion</td>
<td>HIV-1 gp41</td>
<td>Albuvirtide$^d$</td>
<td>FB006M</td>
<td>Chongqing Frontier Biotechnologies</td>
<td>[146]</td>
</tr>
<tr>
<td><strong>Phase II</strong></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>INSTI</td>
<td>HIV-1 IN</td>
<td>Cabotegravir$^f$</td>
<td>GSK1265744</td>
<td>GlaxoSmithKline</td>
<td>[147]</td>
</tr>
<tr>
<td>Entry</td>
<td>CXCR4 receptor</td>
<td>AMD-070</td>
<td>AMD-11070</td>
<td>Genzyme Corporation</td>
<td>[148]</td>
</tr>
<tr>
<td>Entry</td>
<td>CCR5 receptor</td>
<td>Cenicriviroc</td>
<td>TBR-652; TAK-652</td>
<td>Tobira Therapeutics</td>
<td>[149]</td>
</tr>
<tr>
<td>Entry</td>
<td>CCR5 receptor</td>
<td>PRO-140$^g$</td>
<td></td>
<td>Progenics Pharmaceuticals; CytoDyn Inc</td>
<td>[150]</td>
</tr>
<tr>
<td>Entry</td>
<td>CCR5 receptor</td>
<td>PF-00232798</td>
<td></td>
<td>Viiv Healthcare</td>
<td>-</td>
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<tr>
<td>Entry</td>
<td>CCR5 receptor (gene)</td>
<td>SB-728-T</td>
<td>CCR5-specific zinc finger nuclease</td>
<td>Sangamo BioSciences</td>
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<td>PI Maturation</td>
<td>HIV-1 Gag</td>
<td>TMC-310911</td>
<td>ASC-09</td>
<td>Tibotec/Janssen; Asclelis</td>
<td>[151-152]</td>
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<tr>
<td>PI Maturation</td>
<td>HIV-1 Gag</td>
<td>BMS-955176</td>
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<td>Bristol-Myers Squibb</td>
<td>-</td>
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<td><strong>Phase I</strong></td>
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<td></td>
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<tr>
<td>NNRTI</td>
<td>HIV-1 RT</td>
<td>KM-023</td>
<td></td>
<td>Kainos Medicine</td>
<td>[153]</td>
</tr>
<tr>
<td>INSTI</td>
<td>HIV-1 IN</td>
<td>MK-2048$^g$</td>
<td></td>
<td>Merck &amp; Co</td>
<td>[154-155]</td>
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<tr>
<td>NCINI</td>
<td>HIV-1 IN</td>
<td>GS-9883</td>
<td>BI-224436</td>
<td>Boehringer Ingelheim; Gilead</td>
<td>[118-119]</td>
</tr>
<tr>
<td>Maturation</td>
<td>HIV-1 Gag</td>
<td>GSK 2838323</td>
<td></td>
<td>GlaxoSmithKline</td>
<td>-</td>
</tr>
</tbody>
</table>

$^a$As of 18 June, 2015, [http://www.clinicaltrials.gov](http://www.clinicaltrials.gov) and company press releases available publicly online; $^b$HIV prevention (intravaginal ring) currently under evaluation in the Phase III “ASPIRE” Trial and “The Ring Study”; $^c$CD4-specific humanized IgG4 monoclonal antibody; $^d$synthetic peptide; $^e$humanised anti-CCR5 monoclonal antibody; $^f$For both Treatment (oral) and HIV prevention (long-acting injectable packaged into nanoparticles); $^g$HIV prevention (intravaginal ring) in formulation with Vicriviroc; $^h$Announcement that funding for Phase III trial has been raised (July 2014); NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.
Table 2. New classes of HIV-1 RT and IN inhibitors described in the literature but not yet in clinical trials or clinical use.

<table>
<thead>
<tr>
<th>Drug Class</th>
<th>Inhibitory Mechanism</th>
<th>Description</th>
<th>Example Inhibitors</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HIV-1 REVERSE TRANSCRIPTASE (RT)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nucleotide-competiting RT Inhibitors (NcRTI)</td>
<td>Allosteric</td>
<td>Compete with nucleotides for RT binding</td>
<td>indolopyridones e.g. INDOPY-1 benzo[4,5]furo[3,2,d]pyrimidin-2-one series DAVP-1</td>
<td>[156-159]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[160-161]</td>
</tr>
<tr>
<td>RNase H inhibitors</td>
<td>Active Site</td>
<td>Complex with the divalent metal cofactor (Mg$^{2+}$ or Mn$^{2+}$) required for catalysis</td>
<td>N-hydroxyimides hydroxylated tropolones e.g. β-thujaplicinol pyrimidinol carboxylic acids naphthyridiones nitrofuran-2-carboxylic acid carbamoylmethyl esters (NACMEs).</td>
<td>[162-164] [165-167] [168-169] [170] [171-172]</td>
</tr>
<tr>
<td></td>
<td>Allosteric</td>
<td>Inhibit RNase H activity by binding to a secondary site</td>
<td>N-acyl hydrazones thiohene-3-carboxamides thienopyridinidones naphthyridiones dihydroxycoumarins e.g. F3284-8495 (33)</td>
<td>[173-175] [176-177] [178] [179] [180]</td>
</tr>
<tr>
<td>RT dimerization inhibitors</td>
<td>Allosteric</td>
<td>Stabilise or destabilize RT dimer by binding at p66/p51 interface</td>
<td>peptide-based inhibitors e.g. P$_{AW}$, Pep-7 TSAO-T$^b$ BBNH</td>
<td>[181-183]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[184-185] [186]</td>
</tr>
<tr>
<td>T/P inhibitors</td>
<td>Allosteric</td>
<td>Inhibits T/P binding</td>
<td>APEX57219 2GP SY-3E4 KM-1</td>
<td>[104] [187] [102] [103]</td>
</tr>
<tr>
<td>Dual inhibitors</td>
<td></td>
<td>Inhibits polymerase and RNase H activities</td>
<td>anthraquinones propenones</td>
<td>[188]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[189-190]</td>
</tr>
<tr>
<td><strong>HIV-1 INTEGRASE (IN)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allosteric IN Inhibitors (ALLINI/LEDGIN)</td>
<td>Allosteric</td>
<td>Multi-modal: inhibit LEDGF binding to IN LEDGF/p75 binding pocket affecting IN multimerisation, LEDGF binding and virion maturation</td>
<td>quinolines &amp; quinolones peptides</td>
<td>[112, 191]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[137, 192-193]</td>
</tr>
<tr>
<td>Multimerisation Selective Inhibitors</td>
<td>Allosteric</td>
<td></td>
<td>tetra-acetylated inhibitor</td>
<td>[194]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[139]</td>
</tr>
<tr>
<td>Dual inhibitors</td>
<td>Allosteric</td>
<td>Inhibit both strand transfer and the LEDGF/IN interaction distinct allosteric site from ALLINI **</td>
<td>diketo acids</td>
<td>[195]</td>
</tr>
<tr>
<td>Non-catalytic site IN Inhibitors (NCINI)**</td>
<td>Allosteric</td>
<td></td>
<td></td>
<td>[119]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>RT/IN DUAL INHIBITORS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[196-201]</td>
</tr>
</tbody>
</table>

$^a$ Novel sites or inhibitors identified by fragment screening are not listed in this table and described in Sections 4.3-4.5, 5.3-5.5; $^b$ TSAO-T binds to the NNRTI-binding pocket [184], but has a mechanism and chemical structure distinct from other inhibitors classed as NNRTIs. $^c$ An NCINI, BI 224436, has recently entered Phase I clinical trials (2014).
Table 3. Compounds discovered by fragment-based drug discovery that bind to and/or inhibit HIV-1 reverse transcriptase (RT).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Drug class/binding site</th>
<th>IC₅₀ (µM)</th>
<th>RT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NNRTI (NNIBP)</td>
<td>4</td>
<td>LEᵇ</td>
</tr>
<tr>
<td>2</td>
<td>NNRTI-adjacent</td>
<td>350</td>
<td>0.34</td>
</tr>
<tr>
<td>3</td>
<td>Incoming Nucleotide binding site</td>
<td>200</td>
<td>0.31</td>
</tr>
<tr>
<td>4</td>
<td>Knuckles</td>
<td>No inhibition at 1mM</td>
<td>N/A</td>
</tr>
<tr>
<td>5</td>
<td>Knuckles</td>
<td>600</td>
<td>0.37</td>
</tr>
<tr>
<td>6</td>
<td>Knuckles</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>7</td>
<td>RNase H adj. grip</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>8</td>
<td>ND</td>
<td>259</td>
<td>0.42</td>
</tr>
<tr>
<td>9</td>
<td>NcRTI</td>
<td>222</td>
<td>0.43</td>
</tr>
<tr>
<td>10</td>
<td>T/P competing &amp; RNase H inhibitor</td>
<td>222</td>
<td>0.51</td>
</tr>
<tr>
<td>11</td>
<td>T/P competing</td>
<td>70</td>
<td>0.58</td>
</tr>
</tbody>
</table>

ⁿFragment 1 is from the SPR fragment screen [95], fragments 2-7 were discovered by X-ray crystallographic fragment screening [98] and fragments 8-10 by STD-NMR screening [100]. LE, ligand efficiency, values as published in Geitmann et al [95] for 1, Bauman et al [98] for 2-7, and calculated for fragments 8-11 using the formula LE = -R(T)(IC₅₀)/HAC where R is the gas constant, T is temperature and HAC is the heavy atom, or non-hydrogen, atom count ND, not determined; NNRTI, non-nucleoside reverse transcriptase inhibitor; NNIBP, NNRTI binding pocket; NcRTI, nucleotide-competing reverse transcriptase inhibitor; T/P, template/primer.
Table 4. Compounds discovered by fragment-based drug discovery that bind to and/or inhibit HIV-1 integrase (IN).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Drug class/binding site</th>
<th>$K_d$ (µM)</th>
<th>$IC_{50}$ (µM)$^c$</th>
<th>LE$^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>FBP</td>
<td>2800</td>
<td>ND</td>
<td>0.3</td>
</tr>
<tr>
<td>13</td>
<td>FBP</td>
<td>5500</td>
<td>ND</td>
<td>0.32</td>
</tr>
<tr>
<td>14</td>
<td>FBP</td>
<td>170</td>
<td>ND</td>
<td>0.31</td>
</tr>
<tr>
<td>15</td>
<td>Y3 site</td>
<td>259</td>
<td>295</td>
<td>0.21</td>
</tr>
<tr>
<td>16</td>
<td>Y3 site</td>
<td>ND</td>
<td>5</td>
<td>0.24</td>
</tr>
<tr>
<td>17</td>
<td>ALLINI</td>
<td>1570</td>
<td>ND</td>
<td>0.17</td>
</tr>
<tr>
<td>18</td>
<td>ALLINI</td>
<td>1375</td>
<td>200</td>
<td>0.17</td>
</tr>
<tr>
<td>19</td>
<td>ALLINI</td>
<td>7.6</td>
<td>8.1</td>
<td>0.16</td>
</tr>
</tbody>
</table>

$^a$Compounds 12-14 are from the Wielens et al [128] NMR fragment screen, compound 15-16 were discovered by STD-NMR/SPR fragment screening [130] and fragments 17-19 were described in Peat et al [136];$^b$See original studies for construct of IN central core domain (IN CCD) that was used for each assay; $^c$Inhibitory concentrations at 50% (IC50) values refer to inhibition of LEDGF binding to HIV-1 IN for ALLINIs, determined using the AlphaScreen [137]; $^d$LE, ligand efficiency, either as published for 15, or calculated for compounds 12-14, 16-19 using the equation LE = ($-RT\ln K_d$)/(HAC) where R is the universal gas constant, T is temperature, “HAC” is the heavy atom or non-hydrogen atom count and $K_d$ is the dissociation constant; ND, not determined; FBP, fragment binding pocket; ALLINI, allosteric Integrase inhibitor that binds to the LEDGF interaction site on HIV-1 integrase.
Figure 1. Three new allosteric inhibitory pockets in HIV-1 RT identified using FBDD [98]. (A) Crystal structures of HIV-1 RT:rilpivirine with fragment 2 (Table 3), green, bound in the NNRTI-adjacent pocket (PDB code 4KFB), p66 subunit in blue and p51 in grey, and HIV-1 RT:rilpivirine (PDB code 2ZD1), red. Rilpivirine bound in the NNRTI pocket is shown in yellow. (B) Crystal structures HIV-1 RT:rilpivirine with fragment 3 (Table 3), green, bound in the Incoming Nucleotide Binding Site (PDB code 4ICL), blue, superimposed on HIV-1 RT with rilpivirine only (PDB code 2ZD1), red. (C) Crystal structures HIV-1 RT:rilpivirine with fragment 6 (Table 3), green, bound in the Knuckles pocket (PDB code 4IG3), p66 subunit in blue and p51 in grey, and HIV-1 RT:rilpivirine with fragment 4 (Table 3), yellow, also bound in the Knuckles pocket (PDB code 4IFY), light orange. Side chains are shown in A, B and C for residues interacting with bound fragment molecules. Images were generated using Pymol (Schrödinger).
Figure 2. **Fragment-based discovery of inhibitory fragments of HIV-1 IN central core domain** [128, 136]. (A) *left panel*, crystal structure of HIV-1 IN$_{CCD}$, dark green, bound to compound 12 (Table 4) (PDB code 3OVN) showing the interaction with Ser195 via a key water molecule (red) in the fragment binding pocket (FBP); *right panel*, crystal structure of HIV-1 IN$_{CCD}$, orange, bound to optimised compound 14 (Table 4) (PDB code 3AO2) showing a direct interaction with Ser195 in the FBP. (B) Crystal structure of HIV-1 IN$_{CCD}$, dark green, bound to compound 15 (Table 4) (PDB code 3NF6), and key interacting residues His183, Gln62 and Ser147.