Letter

Development of a Raltegravir-based Photoaffinity Labeled Probe for Human Immunodeficiency Virus-1 Integrase Capture

Nicolino Pala, Francesca Esposito, Enzo Tramontano, Pankaj Kumar Singh, Vanna Sanna, Mauro Carcelli, Lisa D. Haigh, Sandro Satta, and Mario Sechi

ACS Med. Chem. Lett., **Just Accepted Manuscript** • DOI: 10.1021/acsmedchemlett.0c00009 • Publication Date (Web): 28 Apr 2020

Development of a Raltegravir-based Photoaffinity Labeled Probe for Human Immunodeficiency Virus-1 Integrase Capture

Nicolino Pala,[†] Francesca Esposito,[‡] Enzo Tramontano,[‡] Pankaj Kumar Singh,[†] Vanna Sanna,[§] Mauro Carcelli,[#] Lisa D. Haigh,[°] Sandro Satta,[^,||] and Mario Sechi[†,*]

[†]Department of Chemistry and Pharmacy, Laboratory of Drug Design and Nanomedicine, University of Sassari, Italy; [‡]Department of Life and Environmental Sciences, University of Cagliari, Italy; [§]Nanomater s.r.l., c/o Porto Conte Ricerche, Alghero, Italy; [#]Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Italy; [°]Department of Chemistry, Imperial College London, UK; [^]Centre for Pharmacology & Therapeutics, Department of Medicine, Imperial College London, UK; [||]Department of Medicine, David Geffen School of Medicine, University of California, Los Angeles, USA

KEYWORDS: HIV-1 Integrase Inhibitors; Photoaffinity labeling; Raltegravir; Molecular recognition.

ABSTRACT: Photoaffinity labeling (PAL) is one of the upcoming and powerful tools in the field of molecular recognition. It includes determination of dynamic parameters, such as identification and localization of the target protein and the site of drug binding. In this study, a photoaffinity labelled probe for full length human immunodeficiency virus-1 integrase (HIV-1 IN) capture was designed and synthesized, following the structure of the FDA-approved drug Raltegravir. This photoprobe was found to retain the HIV-IN inhibitory potential in comparison to its parent molecule, and demonstrates ability to label the HIV-1 IN protein. Putative photoprobe/inhibitor binding sites near to the catalytic site were then identified after protein digestion coupled with mass and molecular modelling analyses.

A prerequisite for structure-based drug design (SBDD) is an understanding of the principles of molecular recognition in protein-ligand complexes.[1,2] Experimental and computational techniques such as X-ray-crystallography, biochemical assays, and molecular modelling are well validated tools in providing structural details at molecular and atomic level.[1,3] Each of these methods possesses peculiar characteristics with advantages and limitations, thus the management of complementary data constitutes a major goal for a better understanding of biochemical and drug binding mechanisms.

Photoaffinity labeling (PAL), i.e., the "capture" of proteins by small molecules via photoactivation mediated irreversible cross-linking, sequentially analyzed by mass spectrometry, represents a powerful method for identifying sites of molecular interactions.[4] PAL is based on the ability of some functional groups (photophores) to form reactive species which are able to create covalent bonds if irradiated with particular wavelengths.[5-8]

In PAL, a photophore is incorporated into the structure of a ligand capable of interacting with a particular target. The photoprobe thus obtained will selectively establish interactions with its putative binding site and, following a procedure called "photoactivation", will form irreversible covalent bonds (photo linkage) with adjacent amino acid residues (Figure 1).[5,6]

The choice of the photophore is a critical point for the PAL. This chemical entity must satisfy various criteria such as sufficient affinity for the target, reduced dimensions, chemical stability, photoactivation at wavelength that does not compromise the structural integrity of the target, as well as the ability to easily form a single covalent bond. Moreover, an optimal activity of the photoprobe (i.e. within the same order of magnitude as the inhibitor used as a model), is required for a successful PAL experiment. However, in some cases, it has been possible to use photoprobe with markedly lower activities than the "ancestor compounds", and compounds with as much as 100 times lower activity can still be useful.

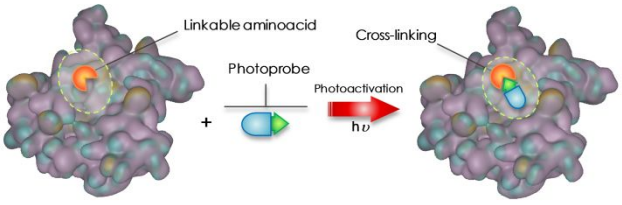


Figure 1. Schematic concept of cross-linking by photoprobe with a "linkable" amino acid.

Among various photoreactive groups available for photoprobe generation, compounds containing a benzophenone chemotype demonstrate some advantages in the

study of protein structures and their interactions.[10] By exploiting the highly electrophilic character of the carbonyl in the excited state, they are able to react also with generally inert bonds. Moreover, the greater chemical stability in normal conditions, compared to other photophores such as azides and diaziridines, allows benzophenone derivatives to be manipulated even in the presence of ambient light, without running the risk of triggering protein degradation processes.

As a suitable model for a PAL investigation, we chose the enzyme HIV-1 integrase (HIV-1 IN), which is a key viral enzyme in HIV replication that catalyzes the insertion of retro-transcribed viral cDNA into the host genome to form a stable provirus.[11-13] Because of its role in HIV cell cycle, and considering that it does not have any host cellular homolog, HIV-1 IN has been validated as a target for antiretroviral drug development.[14,15] First-generation HIV-1 IN inhibitors such as Raltegravir (RAL, Chart 1), Elvitegravir and the second-generation inhibitor Dolutegravir, followed by Cabotegravir and Bictegravir, evolved from the β-diketo acids (DKA) pharmacophore.[15-17]

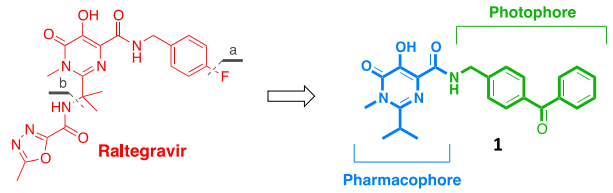


Chart 1. The structures of drug Raltegravir and designed photoprobe.

All the developed inhibitors predominantly inhibit the strand transfer (ST) step by acting as chelating functional group, required to chelate two Mg^{2+} metal ions present in the active site of catalytic core domain (CCD).[18-22] Although the addition of an HIV-1 IN inhibitor to existing components of antiretroviral therapy has improved the outcome of highly active antiretroviral therapy (HAART) by potential synergism, there is still a significant need to acquire new and more reliable information detailing the interaction between IN inhibitors and the binding site, possibly by exploring mechanistic behavior in a "dynamic" condition.

Recently, we and others have reported the incorporation of photoreactive groups into DKA-based HIV-1 IN inhibitors, and these agents can be used to identify the DKA binding site on the IN protein.[23-25] Nevertheless, due to the relative lability of the 2-hydroxy-4-oxobut-2-enoic motif, typical DKAs were found unsuitable for advanced stage of PAL analyses. Again, PAL approach and mass spectrometry analysis were successfully used to identify a unique HIV-1 IN inhibitor-binding site for a series of coumarin-containing IN inhibitors.[26] Therefore, although many structural X-ray crystallographic structures are available to elucidate the interaction between HIV-1 IN inhibitors within the active site,[27-29] further insights could be useful for clarifying the binding mode of HIV-1 IN inhibitors through determination of dynamic parameters, as well as to identify other sites of action.[14, 15,17, 30-32]

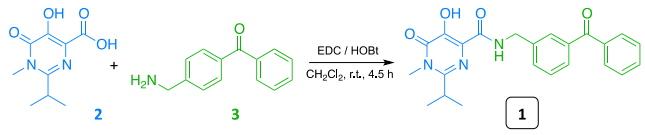
In this scenario, we sought to design and synthesize a novel photoaffinity probe (**1**, Chart 1), structurally related to the drug RAL, that could effectively label the viral full length HIV-1 IN, which could be further used for extensive PAL studies.

After the preparation of the photoprobe, both western-blotting (WB) analyses coupled with mass spectrometry were performed to prove effective linkage of **1** to the target protein. Then, protein digestion, mass experiments and computational modelling, i.e. docking and molecular dynamics, helped us to rationalize the behavior of the putative probe-HIV-1 IN interactions.

Specifically, considering the greater chemical stability, a photoactivable benzophenone group as a photophore was introduced in the carboxamide side chain of the pyrimidine scaffold in RAL, in place of the *p*-F-benzyl moiety. Moreover, for molecular simplification purpose, the heterocyclic substituent was removed from the isopropyl substituent (Chart 1, a and b, respectively).

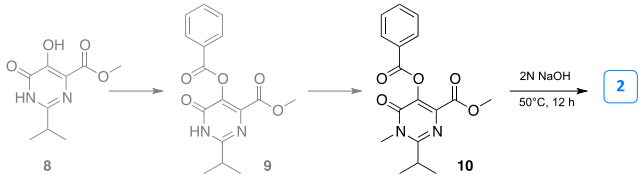
The preparation of desired photoprobe **1** was achieved by following the synthetic approach previously reported by us for the preparation of the RAL-based analogue HL[2],[33,34] with some modifications. In particular, **1** was obtained by amidation of the "synthons" **2** and **3** in the presence of N-(3-dimethylpropyl)-N'-ethylcarbodiimide hydrochloride (EDC) and 1-hydroxybenzotriazole hydrate (HOBt), in dichloromethane (CH_2Cl_2) at room temperature (Scheme 1).

Scheme 1. Preparation of photoprobe 1.



The key synthones **2** and **3** were synthesized by two separate synthetic routes, as outlined in Scheme 2 and in Supporting Information.

Scheme 2. Synthetic routes for the preparation of the synthon 2.



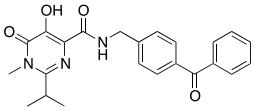
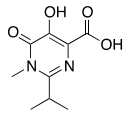
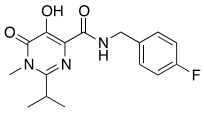
Briefly, the key N-methyl precursor **10** was obtained in good yields by sequential protection of the hydroxyl function in position 5 of dihydroxypyrimidine derivative **8**, by reaction with benzoic anhydride in pyridine, with the formation of the intermediate **9**, which was selectively methylated at the N1 with methyl iodide and Cs_2CO_3 in anhydrous tetrahydrofuran (THF) (Scheme 2, see in Supporting Information for experimental details).[33] Alkaline hydrolysis of the ester **10** gave the dihydropyrimidine carboxylic acid **2**.

The preparation of the (commercially available) aminodiphenylketone **3** was accomplished by starting from the

4-methylbenzophenone **11** (Scheme 3, Supporting Information), following a procedure previously reported by us.[33] All compounds have been characterized by means of NMR, IR and mass spectrometry.

Next, the photoprobe **1** was tested for its ability to inhibit the overall HIV-1 IN integration in *in vitro* assays, employing purified enzyme, and using RAL and HL[2] as reference compounds (Table 1). In order to define the minimal chemical features of **1** required for inhibitory activity, the synthon **2**, without the benzyl moiety, was used as a control.

Table 1. *In vitro* HIV-1 integrase inhibition assay.

Cpds	Structure	[a]IC_{50} IN LEDGF-independent integration (μM)
1		2.3 ± 0.4
2		73.0 ± 2.0
[b]**HL[2]**		0.14 ± 0.03
Raltegravir		0.058 ± 0.02

[a]Compound concentration required to inhibit the HIV-1 IN catalytic activities by 50% in the absence of LEDGF. [b]Data from ref. 33.

The results indicated that the photoprobe **1** was able to inhibit the HIV-1 IN catalytic activity, with an IC_{50} value of 2.3±0.4 μM, even if resulted about 16-fold less potent of HL[2] and about 40-fold less effective with respect of the RAL (IC_{50s} = 2.3±0.4 μM *vs* IC_{50} = 0.058±0.02, for **1** and RAL, respectively). Furthermore, the acid intermediate **2** (which did not bear any aromatic substituent in position 4) seems to retain a residual inhibitory activity (IC_{50} = 73.0±2.0). These results confirm that the nature of the substituents in the carboxamide side chain of the pyrimidine ring significantly influence the potency of the pharmacophoric scaffold. However, the probe **1** retains an activity in the low micromolar range, thus making it suitable to be used for micro-level PAL analysis.

Next, **1** was submitted to crosslinking experiment, where it was illuminated at 360 nm (UV-cross linking) in the presence of full-length HIV-1 IN enzyme (see the amino acid sequence in Supporting information), then subjected to polyacrylamide gel electrophoresis (SDS-PAGE gel). Exposure of the gel to UV-transillumination showed clear fluorescence bands at the expected region (i.e. ~35 kDa), corresponding to labeled IN protein, further confirmed by staining. Figure 2A shows a representative picture of gel electrophoresis analysis demonstrating the capture of HIV-1 IN by the photoprobe, thus supporting the ability of **1** to irreversibly conjugate the enzyme protein.

After capture of the protein, the enzyme-protoprobe (HIV-1 IN + **1**) complex was directly investigated by mass spectrometry (MS) experiments (see LC/MS traces of free and labeled proteins in Figure 2B). Considering the MW of the free HIV-1 IN full protein (MS spectrum of HIV-1 IN alone is shown in Figure 2C), the analyses of the ESI-MS spectra of the incubated photoreacted sample revealed that the molecular weight shift would correspond to one (Figure 2D) or two molecules (Figure 2E) of photoprobe **1**. It is worth noting that the stoichiometric ratio of HIV-1 IN full protein to the photoprobe should be 1:1 or 1:2, or more (other peaks could be revealed), depending from the protein:photoprobe ratio before photoactivation, an excess of probe (i.e. from 1:1.5 to 1:5 ratio) can lead to multiple labeling.

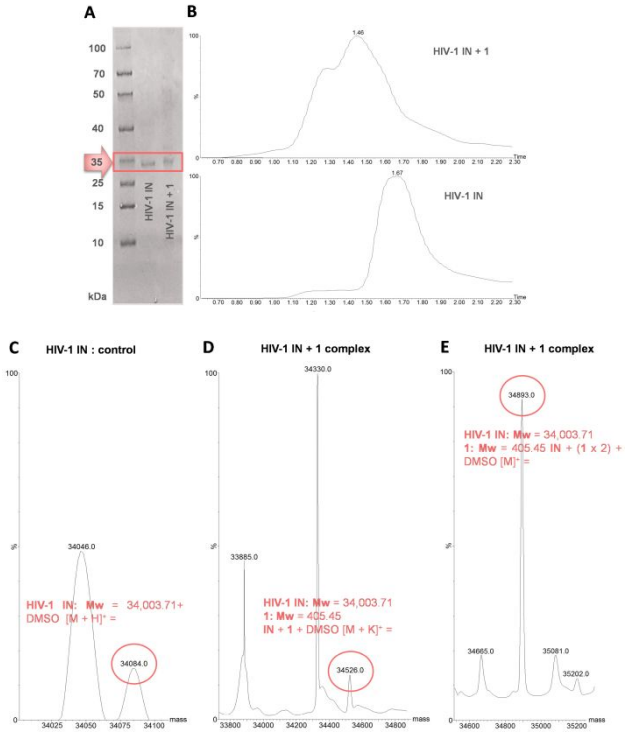


Figure 2. A) Results of gel electrophoresis analysis of capture of HIV-1 IN by the photoprobe **1**. Lines showed both the HIV-1 IN alone, and the mixture HIV-1 IN + photoprobe **1**, after UV irradiation at 360 nm. B) LC/MS total-ion current (TIC) traces showing distinct shift in retention time between the HIV-1 IN alone (bottom) and the protein after activation (top) with the probe in molar excess. C) ESI-MS spectrum expansion of the HIV-1 IN free protein. D) Spectrum of the HIV-1 IN + **1** complex incubated in 1:1.5 and E) 1:5 protein:photoprobe ratio, after irradiation.

To investigate which amino acid pattern could be involved in photolinking/interaction by photoprobe/inhibitor with the protein, the HIV-1 IN + **1** complexes were submitted to tryptic digestion, and the total digest and obtained fractions were analyzed by MS experiments. From the predicted peptide fragments suggested by trypsic digestion (see in Supporting Information), we identified ^{164}ELK^{166} (fragment **14**) as potential peptide crosslinked to photoprobe **1** (Figures 3A-D). Curiously, in addition to **14**, another peptide, i.e. ^{42}EIVASCDK^{49} (fragment **5**) can be found after incubation with a molar excess of probe **1**.

Finally, keeping in mind the indications provided from mass analyses, we envisaged to computationally explore the topological space of the enzyme accessible to the probe **1** on the catalytic site.

Specifically, while the identified peptide **14** was located in the CCD, in region 50-212, the fragment **5** was positioned in the N-terminal domains (NTD). Therefore, we focused our exploration on catalytic site, closely to key catalytic amino acid E152. For clarification purpose, the fragment ^{164}ELK^{166} resulted from the digested full protein corresponds to ^{157}ELK^{159} of the regular HIV-IN sequence (see in Supporting Information). According to mass results, **1** would bind to the peptide ^{157}ELK^{159}, located near the catalytic site. Interestingly, along with other residues, this peptide bears the K159, which is known for having important role in DNA binding with the enzyme, and may affect catalytic activity.[35] Additionally, we realized that the other peptide, i.e. ^{35}EIVASCDK^{42} (i.e. ^{42}EIVASCDK^{49} in our digested protein) found after incubation with a molar excess of probe **1**, can be located within the N-terminal domain, and would include an amino acid, i.e. Cys40, involved in the zinc finger motif.

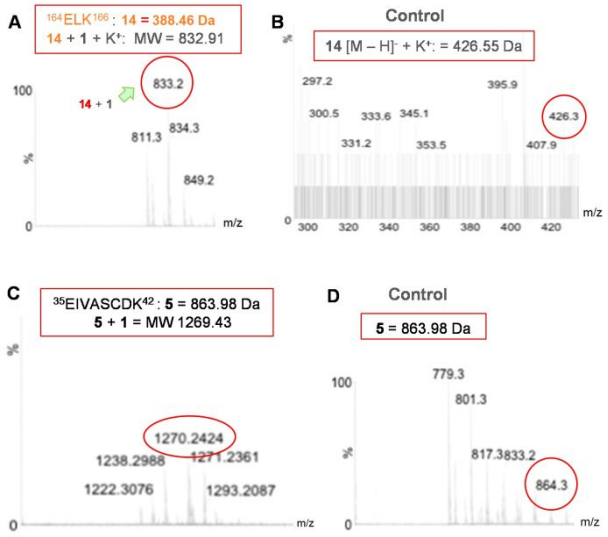


Figure 3. MS spectra of fragments ^{164}ELK^{166} (^{157}ELK^{159} in regular HIV-1 IN), linked to photoprobe **1** (A), its respective free peptide (B), and ^{157}ELK^{159}, ^{35}EIVASCDK^{42} + **1** (C), and the unlabeled peptide (D), from total tryptic digest of labeled HIV-1 IN + **1** conjugated.

We then performed docking and molecular dynamics studies to predict interaction of photoprobe **1** in the IN core domain (PDB: 1QS4).[36]

Docking results indicated that the amino acid residues involved in the binding were as follows: Ser119, Glu92, His67, Thr66, Lys159, Lys156, Glu152, Gly149, Gln48. In particular, the benzophenone moiety of compound **1** occupied an area close to the backbone amino acid residues K156 and K159. Collectively, the disposition of this probe within this amino acid pocket is coherent with the established binding mode of this class of HIV-1 IN inhibitors. The interaction of **1** on the surface of HIV-1 IN core domain is shown in Figure 4A.

Molecular dynamic simulations revealed that the pharmacophoric group on the probe **1** maintained the key interaction responsible for the inhibitory activity (Figure 4B).

RMSD plot disclosed that a stable complex was formed between the photoprobe and HIV-IN, where the carbonyl of the benzophenone (photophoric moiety) and the K159 of the protein backbone aligned in vicinity with the distance of ~5Å after the simulation time period of 2 ns calculations (Figure 4B). This finding suggests the formation of a covalent linkage between the backbone peptide ^{157}ELK^{159} and the benzophenone moiety of **1** upon photoactivation.

In this study, we report on the design and synthesis and a preliminary experimental validation of a RAL-based photoaffinity probe for full length HIV-1 IN capture, to be used in extensive PAL analysis. The developed photoprobe **1** retained IN inhibition potency against purified enzyme with respect to the reference compounds. Analysis of photoactivation experiment as well as data obtained from trypsin digestion coupled with mass experiments seems to prove an efficient capture of HIV-1 IN by the probe, which would establish photolinkage in proximity of the enzyme active site, as also supported by computational modeling. Work is in progress to plan a tandem HPLC-mass spectrometry for a complete PAL procedure.

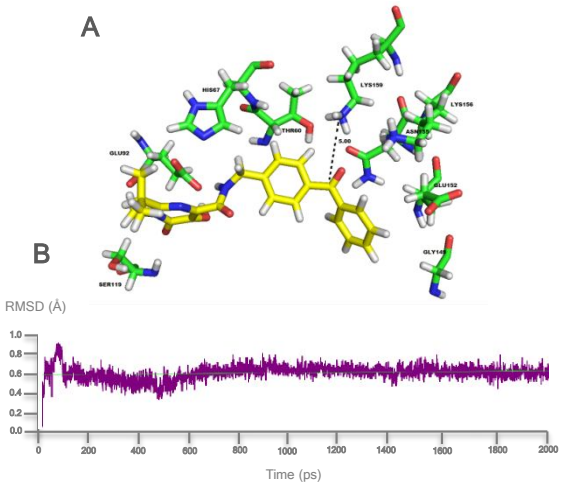


Figure 4. A) Binding mode for photoprobe **1** within the HIV-1 IN catalytic site after MD simulation for 2ns. The residues interacting with the probe **1** are shown. The distance between the Lys159 and the carbonyl group of **1** is also highlighted. B) RMSD from the starting protein structure by MD simulations..

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI:

Experimental methods. Synthesis and characterization details of photoprobe **1**; NMR and Mass spectra of **1**; preparation of the synthons **2** and **3**, and their intermediates **6-10**, **12**, **13**; expression and purification of recombinant IN; HIV-1 IN HTRF inhibition assays; photoaffinity labeling of HIV-1 IN; SDS-page experiments; trypsic digestion of HIV-1 IN; site of cleavage and peptides generated by tryptic digestion; alignment of the HIV-1 IN sequences; molecular modelling. (PDF)

AUTHORS INFORMATION

Corresponding Author

*(M.S.) Phone: +39 079-228-753. Fax: +39 079-229-559. E-mail: mario.sechi@uniss.it.

ORCID:

Nicolino Pala: 0000-0002-9681-5233
Francesca Esposito: 0000-0001-9725-7977
Enzo Tramontano: 0000-0002-4849-0980
Pankaj Kumar Singh: 0000-0002-4774-6358
Vanna Sanna: 0000-0001-9068-349
Mauro Carcelli: 0000-0001-5888-4556
Mario Sechi: 0000-0003-2983-6090

Author Contributions

This work was done through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors have the great pleasure to celebrate the 10th years of the ACS Med. Chem. Letters on the Special Issue "Medicinal Chemistry: From Targets to Therapies". The authors declare no competing financial interest.

ACKNOWLEDGMENT

The authors are grateful to Schrödinger Team for the opportunity to evaluate a trial of the latest version of software Maestro.

ABBREVIATIONS

HIV-1, Human Immunodeficiency Virus-1; IN, Integrase; PAL, Photoaffinity Labeling; HAART, Highly Active Antiretroviral Therapy; CCD, catalytic core domain; RAL, Raltegravir; WB, Western-Blotting; SBDD, structure-based drug design; DKAs, β-diketo acids; cDNA; dexoxyribonuleic acid; ST, strand transfer; SDS-PAGE, Sodium Dodecyl Sulphate - PolyAcrylamide Gel Electrophoresis; LEDGF, Lens epithelium-derived growth factor; RMSD, Root-mean-square deviation.

REFERENCES

1) Neamati, N.; Barchi, J. J., Jr. New paradigms in drug design and discovery. *Curr. Top. Med. Chem.* **2002**, *2*, 211-227
2) Hughes J.P., Rees S., Kalindjian S.B., Philpott K.L. Principles of early drug discovery. *Br. J. Pharmacol.* **2011**, *162*, 1239–1249
3) Cooper, D. R.; Porebski, P. J.; Chruszcz, M.; Minor, W. X-ray crystallography: Assessment and validation of protein-small molecule complexes for drug discovery. *Expert Opin. Drug Discov.* **2011**, *6*, 771–782
4) Smith, E.; Collins, I. Photoaffinity labeling in target- and binding-site identification. *Future Med. Chem.* **2015**, **7**, 159–183
5) Dorman, G.; Prestwich, G. D. Using photolabile ligands in drug discovery and development. *Trends Biotechnol.* **2000**, *18,* 64-77
6) Hatanaka, Y.; Sadakane, Y. Photoaffinity labeling in drug discovery and developments: chemical gateway for entering proteomic frontier. *Curr. Top. Med. Chem.* **2002**, *2,* 271-288
7) Robinette, D.; Neamati, N.; Tomer, K. B.; Borchers, C. H. Photoaffinity labeling combined with mass spectrometric approaches as a tool for structural proteomics. *Expert Rev. Proteomics* **2006**, *3*, 399-408
8) Sadakane, Y.; Hatanaka, Y. Photochemical fishing approaches for identifying target proteins and elucidating the structure of a ligand-binding region using carbene-generating photoreactive probes. *Anal. Sci.* **2006**, *22*, 209-218
9) Brunner, J. New photolabeling and crosslinking methods. *Ann. Rev. Biochem.* **2000,** *18*, 64-77
10) Dorman, G.; Prestwich, G. D. Benzophenone photophores in biochemistry. *Biochem.* **1994,** *33*, 5661-5673
11) Hazuda, D. J. HIV Integrase as a Target for Antiretroviral Therapy Curr. Opin. *HIV AIDS* **2012**, *7*, 383–389
12) Pommier, Y.; Johnson, A. A.; Marchand, C. Integrase inhibitors to treat HIV/AIDS. *Nat. Rev. Drug Discovery* **2005**, *4*, 236– 248
13) Li, X.; Krishnan, L.; Cherepanov, P.; Engelman, A. Structural biology of retroviral DNA integration. *Virology* **2011**, *411*, 194– 205
14) Esposito, F.; Tramontano, E. Past and future. Current drugs targeting HIV-1 integrase and reverse transcriptase-associated ribonuclease H activity: single and dual active site inhibitors. *Antivir. Chem. Chemother.* **2013**, *23*, 129-144
15) Yang Li, Shouyi Xuan, Yue Feng, Aixia Yan. Targeting HIV-1 integrase with strand transfer inhibitors. *Drug Discovery Today* **2015,** *20* (4) , 435-449
16) Hazuda, D. J.; Felock, P.; Witmer, M.; Wolfe, A.; Stillmock, K.; Grobler, J. A.; Espeseth, A.; Gabryelski, L.; Schleif, W.; Blau, C.; Miller, M. D. Inhibitors of strand transfer that prevent integration and inhibit HIV-1 replication in cells. *Science* **2000**, *287,* 646−650
17) Choi, E.; Mallareddy, J. R.; Lu, D.; Kolluru, S. Recent advances in the discovery of small-molecule inhibitors of HIV-1 integrase. *Future Science OA* **2018,** *4* (9) , FSO338.
18) Bushman, F. D.; Craigie, R. Activities of human immunodeficiency virus (HIV) integration protein in vitro: Specific cleavage and integration of HIV DNA *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 1339– 1343
19) Neamati, N. Role of metals in HIV-1 integrase inhibitor design. In: HIV-1 Integrase: Mechanism of Action and Inhibitor Design. Wiley Series in Drug Discovery and Development by Nouri Neamati, **2011**
20) Rogolino, D., Carcelli, M., Sechi, M., and Neamati, N. Viral enzymes containing magnesium: metal binding as a successful strategy in drug design. *Coord. Chem. Rev.* **2012**, *256*, 3063–3086
21) Lesbats, P.; Engelman, A. N.; Cherepanov, P. Retroviral DNA Integration. *Chem. Rev.* **2016**, *116*, 12730−12757
22) Grobler, J. A.; Stillmock, K.; Binghua, H.; Witmer, M.; Felock, P.; Espeseth, A. S.; Wolfe, A.; Egbertson, M.; Bourgeois, M,; Melamed, J.; Wai, J. S.; Young, S.; Vacca, J.; Hazuda, D. J. Diketo acid inhibitor mechanism and HIV-1 integrase: implications for metal binding in the active site of phosphotransferase enzymes. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99,* 6661−6666
23) Zhang, X.; Marchand, C.; Pommier, Y.; Burke, T.R. Jr. Design and Synthesis of Photoactivatable Aryl Diketo Acid-Containing HIV-1 Integrase Inhibitors as Potential Affinity Probes. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1205-2007
24) Zhao, X.Z.; Semenova, E.A.; Liao, C.; Nicklaus, M.; Pommier, Y.; Burke, T.R. Jr. Biotinylated biphenyl ketone-containing 2,4-dioxobutanoic acids designed as HIV-1 integrase photoaffinity ligands. *Bioorg. Med. Chem.* **2006**, *14*, 7816–7825

25) Sechi, M.; Carta, F.; Sannia, L.; Dallocchio, R.; Dessì, A.; Neamati, N. Design, synthesis, molecular modeling and anti-HIV 1 integrase activity of a series of photoactivatable diketo acid-containing inhibitors as affinity probes. *Antiviral Res.* **2009**, *81*, 267-276
26) Al-Mawsawi, L.Q.; Fikkert, V.; Dayam, R.; Witvrouw, M.; Burke, T.R.; Borchers, C.H; Neamati, N. Discovery of a small-molecule HIV-1 integrase inhibitor-binding site *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 10080-10085
27) Maertens, G. N.; Hare, S.; Cherepanov, P. The mechanism of retroviral integration from X-ray structures of its key intermediates. *Nature* **2010**, 468, 326-329
28) Hare, S.; Gupta, S. S.; Valkov, E.; Engelman, A.; Cherepanov, P. Retroviral Intasome Assembly and Inhibition of DNA Strand Transfer. *Nature* **2010**, *464*, 232−236
29) Hare, S.; Vos, A. M.; Clayton, R. F.; Thuring, J. W.; Cummings, M. D.; Cherepanov, P. Molecular mechanisms of retroviral integrase inhibition and the evolution of viral resistance. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *46,* 20057–20062
30) Carcelli, M.; Rogolino, D.; Gatti, A.; Pala, N.; Corona, A.; Caredda, A.; Tramontano, E.; Pannecoque, C.; Naesens, L.; Esposito F. Chelation motifs affecting metal-dependent viral enzymes: N'-acylhydrazone ligands as dual target inhibitors of HIV-1 integrase and reverse transcriptase ribonuclease H domain. *Front. Microbiol.* **2017**, *8*, 1-10
31) Corona, A.; Di Leva, F. S.; Rigogliuso, G.; Pescatori, L.; Madia, V. N.; Subra, F.; Delelis, O.; Esposito, F.; Cadeddu, M.; Costi, R.; Cosconati, S.; Novellino, E.; di Santo, R.; Tramontano E. New insights into the interaction between pyrrolyl diketoacids and HIV-1 integrase active site and comparison with RNase H. *Antiviral Res.* **2016,** *134*, 236-243
32) Esposito, F.; Sechi, M., Pala, N.; Sanna, A.; Koneru, P. C.; Kvaratskhelia, M.; Naesens, N.; Corona, A.; Grandi, N.; di Santo, R.; D'Amore, V. M.; Di Leva, F. S.; Novellino, N.; Cosconati, S.; Tramontano, E. Discovery of dihydroxyindole-2-carboxylic acid derivatives as dual allosteric HIV-1 Integrase and Reverse Transcriptase associated Ribonuclease H inhibitors. *Antiviral Res.* **2020**, *174*, 104671
33) Bacchi, A.; Carcelli, M.; Compari, C.; Fisicaro, E.; Pala, N.; Rispoli, G.; Rogolino, D.; Sanchez, T. W.; Sechi, M.; Sinisi, V.; Neamati, N. Investigating the role of metal chelation in HIV-1 integrase strand transfer inhibitors. *J. Med. Chem.* **2011**, *54*, 8407-8420
34) Gardelli, C.; Nizi, E.; Muraglia, E.; Crescenzi, B.; Ferrara, M.; Orvieto, F.; Pace, P.; Pescatore, G.; Poma, M.; Ferreira Mdel, R.; Scarpelli, R.; Homnick, C. F.; Ikemoto, N.; Alfieri, A.; Verdirame, M.; Bonelli, F.; Paz, O. G.; Taliani, M.; Monteagudo, E.; Pesci, S.; Laufer, R.; Felock, P.; Stillmock, K. A.; Hazuda, D.; Rowley, M.; Summa, V. Discovery and synthesis of HIV integrase inhibitors: development of potent and orally bioavailable N-methyl pyrimidones. *J. Med. Chem.* **2007**, *50*, 4953-4975
35) Krishnan, L.; Li, X.; Naraharisetty, H.L.; Hare, S.; Cherepanov, P.; Engelman, A. Structure-based modeling of the functional HIV-1 intasome and its inhibition. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107,* 15910–15915.
36) Goldgur, Y.; Craigie, R.; Cohen, G. H.; Fujiwara, T.; Yoshinaga, T.; Fujishita, T.; Sugimoto, H.; Endo, T.; Murai, H.; Davies, D. R. Structure of the HIV-1 integrase catalytic domain complexed with an inhibitor: a platform for antiviral drug design. *Proc. Natl. Acad. Sci.* USA **1999**, *96*, 13040-13043.

SYNOPSIS TOC

Graphical representation of the photoactivation of an affinity probes with a linkable amino acid on the target protein. A Raltegravir-based photoaffinity labelled probe has been designed and synthesized for human immunodeficiency virus-1 integrase capture (HIV-1 IN). This photoprobe retained the HIV-IN inhibitory potential in comparison to its parent drug, and labeled the HIV-1 IN protein in proximity of the catalytic site.

Synthesis and Biological Evaluation of a Photoaffinity Labeled Probe for Human Immunodeficiency Virus-1 Integrase Capture