Photocontrollable PROTAC molecules – structure and mechanism of action

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Abstract

Traditional drug discovery strategies are usually focused on occupancy of binding sites that directly affect functions of proteins. Hence, proteins that lack such binding sites are generally considered pharmacologically intractable. Modulators of protein activity, especially inhibitors, must be applied in appropriate dosage regimens that often lead to high systemic drug exposures in order to maintain sufficient protein inhibition in vivo. Consequently, there is a risk of undesirable off-target drug binding and side effects. Recently, PROteolysis TArgeting Chimera (PROTAC) technology has emerged as a new pharmacological modality that exploits PROTAC molecules for induced protein degradation. PROTAC molecule is a heterobifunctional structure consisting of a ligand that binds a protein of interest (POI), a ligand for recruiting an E3 ubiquitin ligase (an enzyme involved in the POI ubiquitination) and a linker that connects these two. After POI-PROTAC-E3 ubiquitin ligase ternary complex formation, the POI undergoes ubiquitination (an enzymatic post-translational modification in which ubiquitin is attached to the POI) and degradation. By merging the principles of photopharmacology and PROTAC technology, photocontrollable PROTACs for spatiotemporal control of induced protein degradation have recently emerged. The main advantage of photocontrollable over conventional PROTACs is the possible prevention of off-target toxicity thanks to local photoactivation.

Keywords: induced protein degradation, PROTAC technology, photocontrollable PROTACs

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**Induced protein degradation: a new approach for drug development**

Although the genomic revolution allowed for finding new relations between certain proteins and diseases (1-4), standard strategies for small-molecule drug development are not always sufficient to exploit those findings. The major issue is the fact that many new biological targets do not have appropriate binding sites which can accommodate small molecules resulting in modulation of the target functions (5). Additionally, intracellular localization of some of those biological targets usually makes them pharmacologically intractable. Hence, the pharmacological effect on intracellular proteins is challenging, and this view can be supported by the fact that there are few approved drugs which target scaffolding proteins, transcription factors and other non-enzymatic proteins localized within cells (6,7). Besides this, strategies for small-molecule drug development based on the occupation of proteins' binding sites may demand high systemic drug exposures to achieve sufficient biological targets occupancy in vivo (8). This increases the risk for pharmacological effects of drugs beyond their primary biological targets and, consequently, the occurrence of adverse effects.

In comparison with standard medicinal chemistry strategies of developing small molecules as modulators of target biomolecule functions, which are usually based on the occupancy theory, small-molecule-induced protein degradation is a new approach which has the potential to affect a greater number of proteins. Changing pharmacological strategy from protein inhibition to protein degradation allows for generating pharmacological effects involving proteins generally considered to be pharmacologically intractable (9,10). Additionally, protein degradation can act concomitantly with the available inhibitor-based therapeutic regimens. It is also important to note that the inhibition of certain cellular pathways can cause upregulation of the target protein, which ultimately leads to the development of tolerance to applied inhibitor (11,12). Thus, induced protein degradation not only reduces the number of active proteins that need to be inhibited, but also resists their compensatory overexpression. Besides that, many biological targets that are pharmacologically tractable also have some scaffolding roles which are unattainable to traditional pharmacological approaches, but still contribute to resistance mechanisms (13-19).

The onset of many diseases is often the consequence of abnormal protein functioning, and traditionally this issue is mostly addressed using occupancy-based pharmacology (Figure 1). It means that applied inhibitor occupies the disease-related protein and blocks its functions. Finally, the longer protein functions are blocked, the greater the clinical benefit achieved. Consequently, as mentioned above, high local inhibitory concentrations of the applied inhibitor need to be achieved and maintained in order to provide an appropriate therapeutic response, which together may lead to off-target binding and side effects (8). An alternative is event-driven pharmacology (Figure 1) where the applied drug triggers an event that ultimately reduces cellular levels of the disease-related protein. Some of these approaches are antisense oligonucleotides, small interfering RNAs (siRNAs) and CRISPR-Cas 9 technology. Although nucleic acid-based
tools can be useful in research, their road to clinical trials is quite difficult. For example, unmodified nucleotides are unstable in serum (20), while modified nucleotides tend to accumulate in the kidney (21,22) and can be immunogenic (23,24). Furthermore, nucleic acid-based agents encapsulated by nanoparticles were captured in the liver, and this attempt to improve their pharmacokinetic properties was unsuccessful (25-28). Additionally, siRNAs can engage off-target mRNA, which unfortunately leads to undesired effects (29-31). It is worth mentioning that the efficacy of nucleic acid-based agents is dependent on the target protein half-life, hence, the impact on long-lived proteins is minor compared to short-lived ones (32).

Figure 1. Occupancy- and event-driven pharmacology models

More recently, Crews et al. (32) have developed a concept that combines the modularity of nucleic acid-based strategies with the pharmacology of small-molecule therapeutics. That approach, based on the concept of event-driven pharmacology, was named “Proteolysis targeting chimera” (PROTAC).
**PROTAC technology and PROTAC molecules**

PROTAC technology uses bifunctional molecules where the one end binds a protein of interest (POI), while the other one recruits cellular quality control mechanisms which afterwards induce protein degradation. Transient binding is sufficient to elicit a biological response and, in contrast to the stoichiometric occupancy of the binding site, proteolysis targeting chimeras (i.e. PROTACs) can perform their pharmacological effect in catalytic quantities. Additionally, compared with the dissociation kinetics of an inhibitor from an active site, target protein degradation requires protein resynthesis and all that provides a kinetic advantage to molecules that induce protein degradation. Furthermore, the ligand does not have to occupy the binding site that affects protein function (e.g. the active site of an enzyme) in order to be a degrader – binding at any suitable region of a biological target could potentially induce its degradation (32). Therefore, it can be concluded that PROTACs might overcome common disadvantages of traditional inhibitors.

PROTACs perform their pharmacological effect (i.e. protein degradation) through the active recruitment of ubiquitin-proteasome system, while protein conjugation with ubiquitin (Ub), a 76 amino acid protein, is essential for regulated protein degradation via 26S proteasome. More precisely, PROTACs are heterobifunctional molecules composed of:

a) POI ligand,

b) E3 ubiquitin ligase ligand (i.e. E3 Recruiting Element),

c) linker that connects those two ligands (Figure 2) (33-37).

![PROTAC molecule schematic](image)

**Figure 2. Schematic representation of a PROTAC molecule**

**Slika 2. Šematski prikaz PROTAC molekula**

Hence, ubiquitin-proteasome system (UPS), outlined in Figure 3, is an essential constituent of overall PROTACs mechanism of action.
The crucial step in PROTACs’ mechanism of action is POI ubiquitination (also known as ubiquitylation or ubiquitinylация). This is an enzymatic post-translational modification in which an isopeptide bond is formed between the carboxyl group of the ubiquitin’s last amino acid – Gly76 and the ε-amino group of the POI’s lysine. Ubiquitination cascade starts with the ubiquitin-activating enzyme (E1) (38) which creates a thioester bond with Ub via an ATP-dependent mechanism. Ub is then transferred to the ubiquitin-conjugating enzyme (E2) (39) and the last enzyme, E3 ubiquitin ligase (E3) (40), specifically binds to the POI and catalyzes the transfer of the activated Ub from E2 to the POI. The reaction can continue with repeated cycles of E2/E3 reactions, which results in multiple Ubs transferred onto the POI. Considering that Ub has seven lysines, Ubs can be linked in multiple different ways, yielding polyubiquitin chains with unique topographies. Finally, polyubiquitinated POI is directed to the 26S proteasome for degradation.

PROTACs operate through the active recruitment of an E3 ubiquitin ligase to tag proteins intended for degradation (Figure 4). They bind a POI with one ligand, while the other one recruits the E3 ubiquitin ligase, thereby forming a ternary complex POI- PROTAC-E3 ubiquitin ligase. The recruited E3 ubiquitin ligase mediates ubiquitin transfer from E2 to the POI, the ternary complex dissociates and the ubiquitinated POI degrades in the 26S proteasome. Given that PROTAC is not degraded in this process, it can be bonded again to undegraded POIs. This enables the ubiquitination and, ultimately, degradation of multiple equivalents of POIs only if the interaction between PROTAC and
POI was not covalent. It can therefore be concluded that PROTACs act sub-stoichiometrically and catalytically (41). Such a mode of action is in contrast with traditional inhibitors, where continuous binding of the drug to the target is necessary to achieve an appropriate biological effect.

**Figure 4.** The mechanism of ubiquitination and subsequent protein of interest degradation by a PROTAC molecule

**Slika 4.** Mehanizam ubikvitinacije i posledične degradacije proteina od interesa PROTAC molekulom

**Photocontrollable PROTACs**

Since the initiation of protein degradation study via PROTACs, several classes of these molecules have been explored. The first-generation PROTACs are peptide-based molecules (42). Although they proved the degradation concept, these molecules have some disadvantages, such as low potency, poor cellular permeability and peptide motifs that can be easily recognized as immunogens (43). On the other hand, second-generation PROTACs are non-peptidic molecules (44), which have been extensively studied and they perform effective degradation of POIs (45). The recent modification of these PROTACs is based on photopharmacology which allows optical control to promote POI degradation. Photopharmacology-based PROTACs can be divided into two classes: photo-caged PROTACs (pc-PROTACs) and photoswitchable PROTACs (photoPROTACs), which will be briefly discussed in the following chapters.
Photo-caged PROTACs (pc-PROTACs)

The first paper about pc-PROTACs was published in 2019 by Xue et al. (46) and it presented a general strategy for the development of pc-PROTACs. Those molecules are actually inactive PROTACs in which a ligand for POI or a ligand for E3 ubiquitin ligase is blocked by photocontrollable bulky groups. These protecting groups should have two important features: one is to prevent the binding of the PROTAC molecule to the POI or E3 ubiquitin ligase, and the other is its easy cleavage using light irradiation, which ultimately gives an active PROTAC molecule (Figure 5).

Figure 5. PROTAC releasing from pc-PROTAC after light irradiation
Slika 5. Oslobađanje PROTAC molekula iz pc-PROTAC molekula nakon Ozračivanja svetlošću

The authors exploited photo-removable 4,5-dimethoxy-2-nitrobenzyl (DMNB) group to dBET1 PROTAC molecule and 1 µM of thus obtained pc-PROTAC1 almost completely degraded Bromodomain-containing protein 4 (BRD4) as POI in Ramos cells (Human Burkitt's lymphoma B cells) after irradiation with maximum degradation efficacy (D_{max}) of 93 % (Figure 6). Furthermore, the in vivo activity of synthesized pc-PROTAC1 was confirmed on a zebrafish model. Additionally, the same group made pc-PROTAC3 and various concentrations of it were used to treat Ramos cells. Only after light irradiation at 365 nm for 3.5 minutes were the levels of Bruton's tyrosine kinase (BTK) as POI significantly reduced in a dose-dependent manner after 18 hours (Figure 6). In conclusion, BRD4 and BTK levels were reduced in a dose-dependent manner and the light-induced degradation of both proteins was influenced by irradiation time.
Naro *et al.* (47) coupled other photocontrollable protecting groups – 7-(diethylamino)coumarin (DEACM) and 6-nitropiperonyloxymethyl (NPOM) with von Hippel-Lindau (VHL) ligand and cereblon (CRBN) ligand, respectively, to obtain pc-PROTACs (Figure 7) which were inactive under dark, but induced degradation of Estrogen-related receptor alpha (ERRα) and BRD4 upon light irradiation. The DEACM group was cleaved from VHL ligand at 365 nm and 402 nm, while the NPOM group was cleaved from CRBN ligand at 365 nm.
Photoswitchable PROTACs (PhotoPROTACs)

Light-induced pc-PROTACs activation is an irreversible process, and because of that the optical control of protein degradation using pc-PROTACs is not a reusable process. Therefore, pc-PROTACs may exhibit a cytotoxic effect caused by continuous degradation of a POI (48). New strategies are being created in order to allow reversible protein degradation, and researchers have recently developed reversible and optical controlled PROTACs using some photoswitchable functional groups.

Pfaff et al. (49) merged the strategies of photopharmacology and small-molecule PROTACs and thus developed the concept of photoswitchable, bistable photoPROTACs which have two configurationally stable Z- and E-azo isomers. The activity of those molecules is controlled using light which induces configurational (Z-/E-photoPROTAC) changes (Figure 8). This is a novel concept for continual spatiotemporal control of induced protein degradation that could prevent off-target toxicity, which is an issue generally seen in pharmacotherapy.
The lead structure for the generation of the photoPROTAC was ARV-771 (50), a highly active Bromodomain and extra-terminal domain (BET) protein degrader. Bistable \textit{ortho}-tetrafluoroazobenzene (\textit{o}-F$_4$-azobenzene) was included as the linker between the ligand for BET protein and the VHL ligand, which gave the photoPROTAC (Figure 9). The molecule thus obtained was stable in both \textit{Z}- and \textit{E}-forms, which can interconvert under photochemical conditions. It means that an active \textit{E}-isomer can be obtained under a one-time exposure to irradiation at 415 nm, which consequently enabled stable ternary complex formation, protein ubiquitination and its proteasomal degradation. Due to the bistable property of \textit{o}-F$_4$-azobenzene moiety, continuous irradiation is not necessary for achieving protein degradation. Conversely, the structure of photoPROTAC was adjusted to an inactive configuration after exposure to irradiation at 530 nm (Figure 9).
The critical difference in linker length between active and inactive PROTACs in many examples is about 3 Å and E-/Z-switch in azobenzenes corresponds to a difference of 3-4 Å in topological distance (49). Therefore, the introduction of a photoswitchable linker enabled reversible control over the topological distance between both ligands. It was seen that the Z-isomer was inactive, because the distance defined by that linker state is too short for ternary complex formation between corresponding proteins. On the other hand, the E-isomer was active, since its length is sufficient for engaging both protein partners, formation of productive ternary complex and BRD2 degradation. A significant degradation of BRD4 was not seen after Z- or E- photoPROTAC treatments, although the parent molecule, ARV-771, could degrade both BRD2 and BRD4 proteins, and with greater potency. The authors proposed hypotheses in order to explain those results. The first is that the photoPROTAC used in experiments has a structural feature that makes it different from ARV-771, and that is a reversed amide bond (Figure 9) between (+)-JQ1 and o-F4-azobenzene moiety. In the end, this shift can be held accountable for a newly gained selectivity of the E-photoPROTAC toward BRD2 over BRD4. The second is that the azobenzene motif increases overall stiffness (51) and that could be the reason for a potential loss of some interactions with BRD4. Also, both reversed amide bond and azobenzene moiety could give an unstable ternary complex and, consequently, inefficient ubiquitination and proteasomal degradation of BRD4.

It is important to note that due to the bistable nature of the o-F4-azobenzene motif, the photostationary state of the photoPROTAC is persistent and thus there is no need for continuous irradiation. After all the above, it can be concluded that photoPROTACs enable reversible on/off switching of protein degradation that is compatible with the environment in cells. Additionally, by enabling spatiotemporal control of induced protein degradation, photoPROTACs have an advantage over photocaging strategies in which active molecules are irreversibly released.

**Conclusion**

The PROTAC technology has great potential as a new modality using the event-driven mode of action. There is a lot of evidence that PROTACs using this alternative mode of action exhibit some advantages over traditional, occupancy-driven pharmacology models. The most notable progress using PROTACs has been noticed after the observation that those molecules perform improved binding and degradation selectivity towards homologous protein families and can also overcome inhibition-induced tolerance mechanisms. Besides that, PROTACs have the potential for treating biological targets which are intractable using traditional inhibitors (52). Furthermore, PROTACs do not have to occupy a specific binding site that directly affects protein function in order to be degraders – binding to any position on a target protein could potentially induce degradation. This advantage could be harnessed by using ligands that simply bind to a biological target and do not necessarily perform inhibition or any other effect (32). It is possible to have a PROTAC where its affinities towards POI or E3
ubiquitin ligase might be in a micromolar range, but the ultimate effect in cells can be seen in a picomolar range (53). Third generation PROTACs, arising from photopharmacology and small-molecule degraders, offer new types of precision therapeutics that might prevent off-target toxicity (48,49). Furthermore, in combination with proteomics techniques, photocontrollable PROTACs offer opportunities for studying downstream effects of signaling pathways (54). Taken together, photocontrollable PROTACs enable the light-irradiation-driven induction of protein degradation using photosensitive groups. This strategy is favorable since it can prevent off-target toxicity due to its spatiotemporal controllability.

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References

1. Campbell J, Ryan CJ, Brough R, Bajrami I, Pemberton HN, Chong IY, et al. Large-scale profiling of kinase dependencies in cancer cell lines. Cell Rep. 2016;14(10):2490-501.
2. Cowley GS, Weir BA, Vazquez F, Tamayo P, Scott JA, Rusin S, et al. Parallel genome-scale loss of function screens in 216 cancer cell lines for the identification of context-specific genetic dependencies. Sci Data. doi: 10.1038/sdata.2014.35.
3. Wang T, Birsoy K, Hughes NW, Krupczak KM, Post Y, Wei JJ, et al. Identification and characterization of essential genes in the human genome. Science. 2015;350(6264):1096-101.
4. Barretina J, Caponigro G, Stransky N, Venkatesan K, Margolin AA, Kim S, et al. The Cancer Cell Line Encyclopedia enables predictive modelling of anticancer drug sensitivity. Nature. 2012;483(7391):603-7.
5. Hopkins AL, Groom CR. The druggable genome. Nat Rev Drug Discov. 2002;1(9):727-30.
6. Lazo JS, Sharlow ER. Drugging undruggable molecular cancer targets. Annu Rev Pharmacol Toxicol. 2016;56:23-40.
7. Jin L, Wang W, Fang G. Targeting protein-protein interaction by small molecules. Annu Rev Pharmacol Toxicol. 2014;54:435-56.
8. Adjei AA. What is the right dose? The elusive optimal biologic dose in phase I clinical trials. J Clin Oncol. 2006;24(25):4054-5.
9. Crews CM. Targeting the undruggable proteome: the small molecules of my dreams. Chem Biol. 2010;17(6):551-5.
10. Overington JP, Al-Lazikani B, Hopkins AL. How many drug targets are there? Nat Rev Drug Discov. 2006;5(12):993-6.
11. Duncan JS, Whittle MC, Nakamura K, Abell AN, Midland AA, Zawistowski JS, et al. Dynamic reprogramming of the kinome in response to targeted MEK inhibition in triple-negative breast cancer. Cell. 2012;149(2):307-21.
12. Visakorpi T, Hytynen E, Koivisto P, Tanner M, Keinänen R, Palmberg C, et al. In vivo amplification of the androgen receptor gene and progression of human prostate cancer. Nat Genet. 1995;9(4):401-6.
13. Hatzivassiliou G, Song K, Yen I, Brandhuber BJ, Anderson DJ, Alvarado R, et al. RAF inhibitors prime wild-type RAF to activate the MAPK pathway and enhance growth. Nature. 2010;464(7287):431-5.
14. Heidorn SJ, Milagre C, Whittaker S, Nourry A, Niculescu-Duvas I, Dhomen N, et al. Kinase-dead BRAF and oncogenic RAS cooperate to drive tumor progression through CRAF. Cell. 2010;140(2):209-21.
15. Pouliakos PI, Zhang C, Bollag G, Shokat KM, Rosen N. RAF inhibitors transactivate RAF dimers and ERK signalling in cells with wild-type BRAF. Nature. 2010;464(7287):427-30.
16. Rauch J, Volinsky N, Romano D, Kolch W. The secret life of kinases: functions beyond catalysis. Cell Commun Signal. 2011;9(1):23.
17. Tan X, Thapa N, Sun Y, Anderson RA. A kinase-independent role for EGF receptor in autophagy initiation. Cell. 2015;160(1-2):145-60.
18. Vivanco I, Chen ZC, Tanos B, Oldrini B, Hsieh WY, Yannuzzi N, et al. A kinase-independent function of AKT promotes cancer cell survival. Elife. doi: 10.7554/eLife.03751.
19. Weihua Z, Tsan R, Huang WC, Wu Q, Chiu CH, Fidler IJ, et al. Survival of cancer cells is maintained by EGFR independent of its kinase activity. Cancer Cell. 2008;13(5):385-93.
20. de Smidt PC, Le Doan T, de Falco S, van Berkel TJ. Association of antisense oligonucleotides with lipoproteins prolongs the plasma half-life and modifies the tissue distribution. Nucleic Acids Res. 1991;19(17):4695-700.
21. Geary RS, Watanabe TA, Truong L, Freiter S, Lesnik EA, Sioufi NB, et al. Pharmacokinetic properties of 2'-O-(2-methoxyethyl)-modified oligonucleotide analogs in rats. J Pharmacol Exp Ther. 2001;296(3):890-7.
22. McMahon BM, Mays D, Lipsky J, Stewart JA, Fauq A, Richelson E. Pharmacokinetics and tissue distribution of a peptide nucleic acid after intravenous administration. Antisense Nucleic Acid Drug Dev. 2002;12(2):65-70.
23. Marques JT, Williams BR. Activation of the mammalian immune system by siRNAs. Nat Biotechnol. 2005;23(11):1399-405.
24. Krieg AM. CpG motifs in bacterial DNA and their immune effects. Annu Rev Immunol. 2002;20:709-60.
25. Dahlman JE, Kauffman KJ, Langer R, Anderson DG. Nanotechnology for in vivo targeted siRNA delivery. Adv Genet. 2014;88:37-69.
26. Whitehead KA, Langer R, Anderson DG. Knocking down barriers: advances in siRNA delivery. Nat Rev Drug Discov. 2009;8(2):129-38.
27. Wilhelm S, Tavares AJ, Dai Q, Ohta S, Audet J, Dvorak HF, et al. Analysis of nanoparticle delivery to tumours. Nat Rev Mater. doi: 10.1038/natrevmats.2016.14.
28. Peer D, Lieberman J. Special delivery: targeted therapy with small RNAs. Gene Ther. 2011;18(12):1127-33.
29. Fedorov Y, Anderson EM, Birmingham A, Reynolds A, Karpilow J, Robinson K, et al. Off-target effects by siRNA can induce toxic phenotype. RNA. 2006;12(7):1188-96.
30. Jackson AL, Bartz SR, Schelter J, Kobayashi SV, Burchard J, Mao M, et al. Expression profiling reveals off-target gene regulation by RNAi. Nat Biotechnol. 2003;21(6):635-7.
31. Qiu S, Adema CM, Lane T. A computational study of off-target effects of RNA interference. Nucleic Acids Res. 2005;33(6):1834-47.
32. Lai AC, Crews CM. Induced protein degradation: an emerging drug discovery paradigm. Nat Rev Drug Discov. 2017;16(2):101-14.
33. Sakamoto KM, Kim KB, Kumagai A, Mercurio F, Crews CM, Deshaies RJ. Protacs: Chimeric molecules that target proteins to the Skp1-Cullin-F box complex for ubiquitination and degradation. Proc Natl Acad Sci U S A. 2001;98(15):8554-9.
34. Burslem GM, Crews CM. Small-molecule modulation of protein homeostasis. Chem Rev. 2017;117(17):11269-301.
35. Cromm PM, Crews CM. Targeted protein degradation: from chemical biology to drug discovery. Cell Chem Biol. 2017;24(9):1181-190.
36. Raina K, Crews CM. Targeted protein knockdown using small molecule degraders. Curr Opin Chem Biol. 2017;39:46-53.
37. Fisher SL, Phillips AJ. Targeted protein degradation and the enzymology of degraders. Curr Opin Chem Biol. 2018;44:47-55.
38. Schulman BA, Harper JW. Ubiquitin-like protein activation by E1 enzymes: the apex for downstream signalling pathways. Nat Rev Mol Cell Biol. 2009;10(5):319-31.
39. Ye Y, Rape M. Building ubiquitin chains: E2 enzymes at work. Nat Rev Mol Cell Biol. 2009;10(11):755-64.
40. Deshaies RJ, Joazeiro CA. RING domain E3 ubiquitin ligases. Annu Rev Biochem. 2009;78:399-434.
41. Bondeson DP, Mares A, Smith IE, Ko E, Campos S, Miah AH, et al. Catalytic in vivo protein knockdown by small-molecule PROTACs. Nat Chem Biol. 2015;11(8):611-7.
42. Sakamoto KM, Kim KB, Kumagai A, Mercurio F, Crews CM, Deshaies RJ. Protacs: chimeric molecules that target proteins to the Skp1-Cullin-F box complex for ubiquitination and degradation. Proc Natl Acad Sci U S A. 2001;98(15):8554-9.
43. Zou Y, Ma D, Wang Y. The PROTAC technology in drug development. Cell Biochem Funct. 2019;37(1):21-30.
44. Schneekloth AR, Pucheault M, Tae HS, Crews CM. Targeted intracellular protein degradation induced by a small molecule: En route to chemical proteomics. Bioorg Med Chem Lett. 2008;18(22):5904-8.
45. Burslem GM, Smith BE, Lai AC, Jaime-Figueroa S, McQuaid DC, Bondeson DP, et al. The Advantages of Targeted Protein Degradation Over Inhibition: An RTK Case Study. Cell Chem Biol. 2018;25(1):67-77.
46. Xue G, Wang K, Zhou D, Zhong H, Pan Z. Light-Induced Protein Degradation with Photocaged PROTACs. J Am Chem Soc. 2019;141(46):18370-4.
47. Naro Y, Darrah K, Deiters A. Optical Control of Small Molecule-Induced Protein Degradation. 2020;142(5):2193-7.
48. Li W, Elhassan RM, Fang H, Hou X. Photopharmacology-based small-molecule proteolysis targeting chimeras: optical control of protein degradation. Future Med Chem. 2020;12(22):1991-3.
49. Pfaff P, Samarasinghe KTG, Crews CM, Carreira EM. Reversible Spatiotemporal Control of Induced Protein Degradation by Bistable PhotoPROTACs. ACS Cent Sci. 2019;5(10):1682-90.
50. Raina K, Lu J, Qian Y, Altiere M, Gordon D, Rossi AMK, et al. PROTAC-induced BET protein degradation as a therapy for castration-resistant prostate cancer. Proc Natl Acad Sci U S A. 2016;113(26):7124-9.
51. Velema WA, Szymanski W, Feringa BL. Photopharmacology: beyond proof of principle. J Am Chem Soc. 2014;136(6):2178-91.
52. Pettersson M, Crews CM. PROteolysis TArgeting Chimeras (PROTACs) - Past, present and future. Drug Discov Today Technol. doi: 10.1016/j.ddtec.2019.01.002.
53. Neklesa TK, Winkler JD, Crews CM. Targeted protein degradation by PROTACs. Pharmacol Ther. doi: 10.1016/j.pharmthera.2017.02.027.
54. Sanchez-Vega F, Mina M, Armenia J, Chatila WK, Luna A, La KC, et al. Oncogenic Signaling Pathways in The Cancer Genome Atlas. 2018;173(2):321-37.
Fotokontrolisani PROTAC molekuli – struktura i mehanizam dejstva

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Kratak sadržaj

Tradicionalne strategije razvoja lekova su obično osvrnute na okupiranje vezujućih mesta koja direktno utiču na funkcije proteina. Stoga se proteini koji nemaju takva vezujuća mesta generalno smatraju farmakološki nedodirljivim. Modulatori aktivnosti proteina, naročito inhibitori, koriste se u režimima doziranja koji često dovode do preterane sistemskе izloženosti leku, a sve u cilju održavanja dovoljne inhibicije proteina in vivo. Posledično, postoji rizik od neželjenog vezivanja leka van svog primarnog mesta dejstva i neželjenih efekata. Nedavno je predstavljena tehnologija dirigovane proteolize (PROteolysis TArgeting Chimera, PROTAC) kao novi farmakološki modalitet koji koristi PROTAC molekule za indukovana degradaciju proteina. PROTAC molekuli su heterobifunkcionalne strukture sačinjene od liganda koji se vezuje za protein od interesa (POI), liganda za regrutovanje E3 ubikvitin ligaze (enzima uključenog u ubikvitinaciju POI) i linkera koji ih povezuje. Nakon formiranja ternarnog kompleksa POI-PROTAC-E3 ubikvitin ligaze, POI podleže ubikvitinaciji (enzimskoj post-translacionoj modificaciji u kojoj se ubikvitin vezuje za POI) i degradaciji. Integriranjem principa fotofarmakologije i PROTAC tehnologije, nedavno su nastali fotokontrolisani PROTAC molekuli za prostorno-vremensku kontrolu indukovane degradacije proteina. Zahvaljujući lokalnoj fotoaktivaciji, glavna prednost fotokontrolisanih nad konvencionalnim PROTAC molekulima je moguća prevencija toksičnosti koja nastaje usled dejstva van primarnog biološkog targeta.

Ključne reči: indukovana degradacija proteina, PROTAC tehnologija, fotokontrolisani PROTAC molekuli