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Pegylation – in search of balance and enhanced bioavailability

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ABSTRACT

In the process of finding better therapeutics, thousands of new molecules are synthesised every day. Many of these can be poorly soluble in water, leading to a potentially promising drug being rejected during testing due to its poor solubility. Polyethylene glycol (PEG) has become known as an excellent modification to remedy this and was initially used to increase circulation time and reduce the immunogenicity of therapeutic proteins. Thus significantly increasing their safety and range of use. Another group of compounds in which significant benefits of pegylation have been seen are photosensitisers. Used in photodynamic therapy, they are often characterised by very high hydrophobicity. Pegylation of their structure significantly increases their affinity for cancer cells and facilitates their penetration through cell membranes. Classical small-molecule drugs can benefit from temporary combinations hydrolysed in the body or very short PEG chains. This approach allows a significant increase in the bioavailability of the drug while avoiding the disadvantages of small molecule pegylation. However, the most common motive for pegylation recently is the creation of drug carriers. Liposomes and nanoparticles make it possible to exploit the advantages of PEG to stabilise their structure and increase circulation time while not modifying the structure of the active compound. Unfortunately, PEGs also have their drawbacks. The first is their high molecular weight range, especially for longer chains, which poses difficulties in purification. Another is the emergence of antibodies directed against PEG. Nevertheless, pegylation is still an up-and-coming method for modifying pharmaceutically active molecules.

Introduction

One of the significant challenges in drug design is the poor solubility of many compounds in water. Organic molecules used in medicine are mainly hydrophobic. However, not all of them require or can benefit from increased solubility. According to the BCS (Biopharmaceutics Classification System), there are four classes of drugs. They are segregated according to their solubility (high vs. low) and intestinal permeability (high vs low). Drugs from class 1 are highly soluble and absorb

well; ideally, all drugs would eventually have such desirable properties. Classes 2 and 3 comprise drugs with either low solubility or low permeability, while class 4 drugs with the weakest parameters have the poorest bioavailability. Most modifications to the structure aimed at increasing their solubility in water rely on introducing hydrophilic functional groups (e.g. hydroxyl, amine) into their structure or giving these molecules an electric charge by introducing quaternary ammonium groups or quickly dissociating groups (such as sulfonyl or carboxyl groups). There are several disadvantages to using this approach. Such hydrophilic compounds are not easily transported across biological membranes, mostly lipid in structure. Therefore, such modification will move a drug from BCS class 2 to class 3, not improving overall bioavailability. Additionally, acidic groups such as carboxyl or sulfonyl groups generally increase water solubility only under primary conditions where they can ionise. One of the solutions to improve this is 'pegylation'. It offers the possibility of increasing the solubility and, consequently, bioavailability of drugs in a wide range of applications. Polyethylene glycol (PEG) consists of units containing alternating ethylene groups and oxygen atoms, giving it an amphiphilic structure. The structure is very advantageous from the point of view of penetration through biological membranes because it has an affinity for both the aqueous environment of body fluids and lipid cell membranes. These properties make it a fascinating modification, as it can result in modified drugs transitioning to a higher BCS class, thereby improving their therapeutic usability. One of the most significant advantages of pegylation is that it can improve solubility and permeability at the same time with the use of only one modification. However, due to the variety of substituents and methods of linking them to active molecules, different drugs require a different approach to this up-and-coming method.

PEG can be attached to the target in many ways (**Figure 1**). Due to their large size and slightly negative charge owing to the oxygen atoms, they can be adsorbed on the surface of nanoparticles. In the case of liposomes, PEG is often incorporated into the membrane by adding it to the mixture during formulation. Alternatively, some of the lipid chains in phospholipids can be exchanged with PEG chains. When modifying molecules, a covalently bonding target with PEG is the preferred method and can be used on its own or as a linker to another molecule or carrier. This mini-review will focus on modification utilizing covalent bonds. There are two possible methods of pegylation of small molecules, permanent attachment of the glycol chain or temporary, as in the case of pro-drugs. Nevertheless, only one is preferred for macromolecules. The irreversible method is most commonly used in protein and photosensitiser modification. It provides stability and structure invariability even after drug administration. Still, unfortunately, it can reduce efficiency compared to the original molecule if the size of the PEG chain is too large. The most commonly used PEG chains contain functional groups that react irreversibly with those in the modified compound. Amine, hydroxyl, or thiol groups are common targets of this reaction. The first can create connections, e.g., amide, urethane, secondary or tertiary amine and imine. On the other hand, the

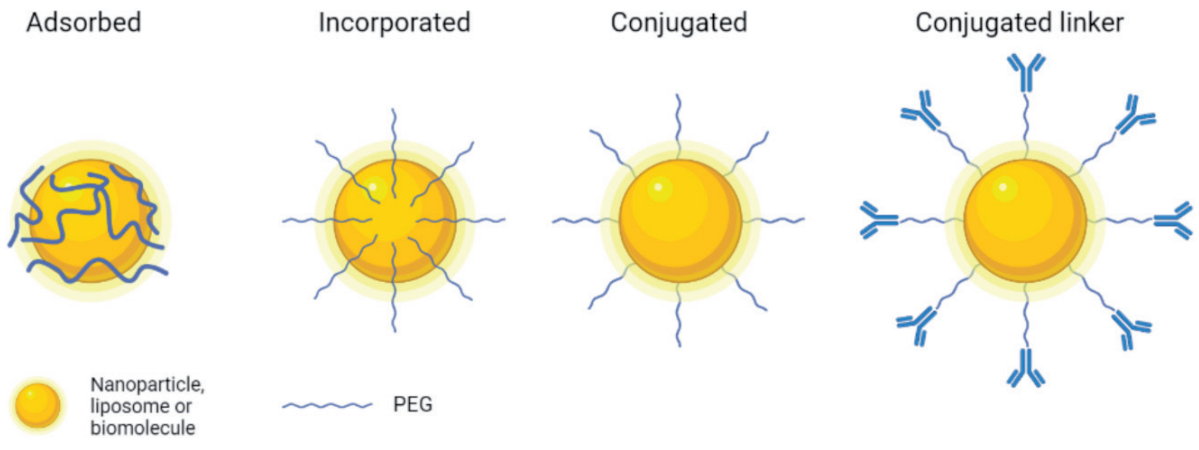

Figure 1. Possible ways of attaching PEG (created with BioRender.com)

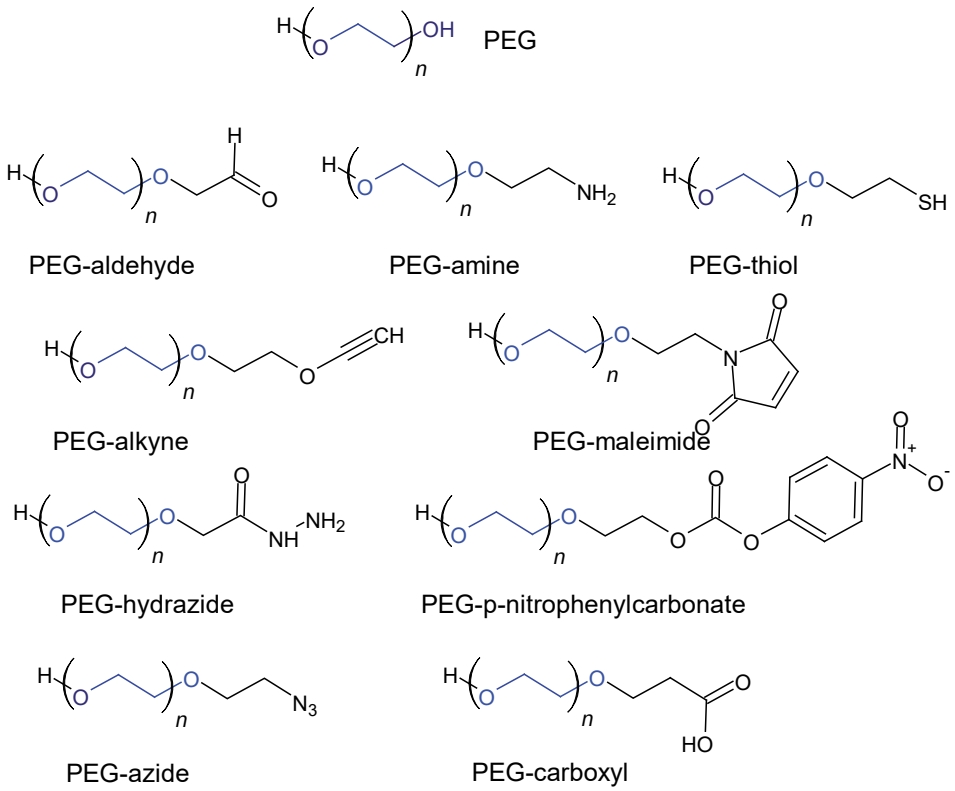

Figure 2. Examples of reagents for introducing PEG chain into molecules

hydroxyl and thiol groups most often form ether and thioether connections. The pro-drug method is based on the temporary attachment of a glycol chain through bonds that undergo gradual hydrolysis (biotransformation) in the patient's body. Ester bonds are mainly used for this, ensuring better solubility and bioavailability at the formulation and administration stage while not reducing the effect of the original molecule. The disadvantage of this approach, however, is the lower stability of such combinations, especially during more extended storage, and the high dependence of the rate of hydrolysis on the patient's conditions [1, 2].

Proteins and enzymes

Until now, pegylation is most often used to modify proteins and enzymes. Several of them have already completed the clinical trial phase and have been approved by the European Medicines Agency (EMA) and US Food and Drug Administration (FDA) (**Table 1**). The primary purpose of protein pegylation is to increase the time they circulate in the body. The pegylated proteins introduced for treatment include interferons used in melanoma and viral hepatitis [3, 4] and filgrastim to manage bone marrow suppression induced by chemotherapy [5]. There are also clinical trials of pegylated enzymes such as arginase and asparaginase in treating leukaemia [6, 7]. PEGylated proteins and enzymes are the groups with the most prominent and heaviest PEG substituents. They usually have a mass of several tens of thousands of Da and are very often branched chains. An additional advantage of protein pegylation is that it usually lowers the immunogenicity of the primary protein, which reduces the body's immune response to the administered drug [4].

Drug carriers

Efforts to increase the bioavailability of drugs have given rise to many forms of drug carriers and delivery systems. The two main groups currently receiving the most attention are liposomes and nanoparticles [15]. At the same time incorporating polyethylene glycol into the structure of drug

Table 1. Pegylated drugs approved by FDA [8–14]

Brand name	Active molecule	Attached PEG	Therapeutical target	Year of approval
Adagen	Adenosine deaminase	5 kDa – multiple chains	Severe combined immunodeficiency disease	1990
Oncaspar	Asparaginase	5 kDa – multiple chains	Acute lymphoblastic leukaemia	1994
Doxil	Liposomal doxorubicin	2 kDa	Ovarian cancer, multiple myeloma	1995
PegIntron	Interferon α-2b	12 kDa	Hepatitis C	2001
Pegasys	Interferon α-2a	40 kDa – branched	Hepatitis C	2001
Neulasta	Filgastrim	20 kDa	Neutropenia	2002
Somavert	Growth hormone receptor antagonist	4–6 x 5 kDa	Acromegaly	2003
Macugen	Anti-VEGF aptamer	40 kDa – branched	Macular degeneration	2004
Mircera	Epoetin-β	30 kDa	Chronic kidney disease-associated anaemia	2007
Cimzia	Anti-TNF Fab	40 kDa – branched	Rheumatoid arthritis and Crohn's disease	2008
Asclera	Dodecyl alcohol	400 Da	Varicose veins	2010
Krystexxa	Uricase	9–11 x 10 kDa	Chronic gout	2010
Sylatron	Interferon α-2b	12 kDa	Melanoma	2011
Omontys	Erythropoietin-mimetic homodimeric peptide	40 kDa – branched	Chronic kidney disease-associated anaemia	2012
Movantik	Naloxone	339 Da	Opioid-induced constipation	2014
Plegridy	Interferon β-1a	20 kDa	Multiple sclerosis	2014
Adynovate	Coagulation factor VIII	20 kDa	Haemophilia A	2015
Rebinyn	Coagulation factor IX	40 kDa	Haemophilia B	2017
Jivi	Coagulation factor VIII	60 kDa	Haemophilia A	2018
Palynziq	Phenylalanine ammonia-lyase	20 kDa	Phenylketonuria	2018
Revcovi	Adenosine deaminase	80 kDa	Adenosine deaminase severe combined immunodeficiency	2018
Asparlas	L-asparaginase	31–39 x 5 kDa	Leukaemia	2018
Udenyca	G-CSF	20 kDa	Infection during chemotherapy	2018
Ziextenzo	G-CSF	20 kDa	Infection during chemotherapy	2019
Esperoct	Coagulation factor IX	40 kDa	Haemophilia A	2019
Nyvepria	G-CSF	20 kDa	Chemotherapy associated neutropenia	2020
Empaveli	Compstatin	40 kDa	Paroxysmal nocturnal hemoglobinuria	2021
Skytrofa	Human growth hormone	4 x 10 kDa	Growth hormone deficiency	2021
Besremi	Interferon α-2b	40 kDa	Polycythemia vera	2021

carriers such as liposomes and nanoparticles is gaining more and more popularity. Such usage does not require modification of the active compound structure. PEG in liposomes is usually an additive to the formulation, while in nanoparticles, it depends on their nature. In the case of metallic nanoparticles, it can be a covalent bond that is very durable, or it can be adsorption based. In PEG liposomes, they most often play a stabilizing and protective role [16]. PEG-containing liposomes significantly improve pharmacokinetic parameters over conventional liposomes and free drugs. PEGs with molar masses in the range of 2–5 kDa are best suited for application [17]. Nawalany et al. have conducted a comparison study in which they test pegylated porphyrin against a non-pegylated compound enclosed in pegylated liposomes. They note that both approaches reduce the dark cytotoxicity but liposomes retain higher activity when exposed to light. The liposomes also internalize the non-pegylated porphyrin into cells faster and in higher concentrations than pegylated porphyrin on its own [18]. The EU and US have approved pegylated liposomal formulation of doxorubicin for treating various cancers for over 25 years. Better pharmacokinetic profile, longer circulation time and lower toxicity are characteristics of the formultion [19]. Pegylation can also significantly improve liposome stability during long-term storage without any negative impact on the delivery of the therapeutic, as shown by Knudsen et al. in their study. Wherein pegylated liposomes con-

taining calcipotriol – a synthetic vitamin D analogue used in psoriasis treatment, were examined and compared with normal ones [20]. Unfortunately, pegylation is not always a perfect solution, as demonstrated during the development of liposomal formulations of vincristine. One of the big problems is its high leakage through the membranes outside the liposomes. It was demonstrated that adding PEG slows down this process, although further studies are necessary for clinical application [21]. Due to their nature, nanoparticles are often accumulated by the phagocytic system and thus removed from circulation.. Aggregation, which can lead to the congestion of capillaries, is still another problem. Therefore, it is necessary to protect them by coating them with various molecules, out of which PEG is the most often used [22]. Covering the surface of nanoparticles prevents aggregation and the response of the phagocytic system. As a result, the time they stay in the bloodstream increases. The most frequently used PEGs for this purpose have a mass of several thousand Da [23]. Pegylation can also increase the circulation time of nanoparticles made from biodegradable polymers like PLGA (poly lactic-co-glycolic acid), an emerging material in controlled drug release systems. Adding PEG to the polymer shields it from hydrolysis and enables copolymer to form micelles for drug transport [24]. Similarly, the nanoparticles made from mesoporous materials have gained much interest recently. Although not fault-free, pegylation can help overcome them. Zhu et al. showed one of the examples in their work on pegylated mesoporous silica nanoparticles as drug carriers. They demonstrate, as many others, better stability and dispersity in aqueous conditions as well as lower toxicity of nanoparticles themselves. Simultaneously, when loaded with doxorubicin, the pegylated carriers exhibit higher cellular uptake and stronger cytotoxic activity against cancer cells than free doxorubicin [25].

PDT and photosensitisers

Photodynamic Therapy (PDT) uses various light-absorbing dyes as photosensitisers (PS) and their ability to produce singlet oxygen or other Reactive Oxygen Species (ROS) to kill cancer cells or pathogenic microorganisms. PS can be applied topically or intravenously, and after it builds up in the cells, it is irradiated with visible light. Illumination activates the photosensitiser, which can then transfer its surplus energy to a molecule such as oxygen and form ROS or directly to the building blocks of cells such as DNA, proteins, and structural elements like membrane, mitochondria and cell walls. The main goal of PDT is to induce oxygen to its reactive singlet state, which then reacts with cellular mechanisms causing damage and leading to apoptosis [26, 27].

Photosensitisers, by their nature, are often compounds with many conjugated double bonds, making them usually highly hydrophobic molecules with poor water solubility. The feature is very unfavourable for its usefulness in photodynamic therapy. One of the main challenges in successful PDT is the even distribution of photosensitiser within a tumour and selectivity against cancer cells. Only a few photosensitisers have been clinically approved, and a few more are in the clinical trial phase [28]. The quaternisation of pyridyl or amino groups, sulfonation, carboxylation and pegylation are typical modifications to increase photosensitisers solubility in water [29]. Another possibility is to enclose photosensitisers in liposomes, which allows them to be dispersed in an aqueous medium. Liposomes can also be modified to increase the ability of compounds to target cancer [16].

One of the more promising modifications of the photosensitiser molecule is pegylation, which links PS to polyethylene glycol chains to increase the water solubility of such conjugates [30]. Other advantages of pegylation include increased stability and better bioavailability. The most common method of photosensitiser pegylation is the permanent introduction of polyethylene glycol chains into the photosensitiser molecule via a stable covalent bond. The vast majority of clinically used photosensitisers have the structure of pyrrole macrocycles [31]. Macrocyclic pyrrole-derived compounds, includes porphyrins, benzoporphyrins, chlorins, porphyrazines, phthalocyanines, naphthalocyanines, corroles and others. These are molecules that usually consist of four pyrrole molecules. The core of these macrocyclic molecules is strongly hydrophobic and mostly soluble in non-polar solvents or insoluble. Numerous solution attempts are being made to

incorporate PEG chains or linkers into their structure. Pavlíčková et al. demonstrated a pegylated derivative of purpurin 18 that exhibits significantly higher photodynamic activity against many cancer cell lines compared to the parent molecule, even with a lower quantum yield of singlet oxygen generation (**Figure 3**) [32]. Zhdanova et al. presented the synthesis of various asymmetric pegylated porphyrins in their work. They show an easy method to obtain many compounds for high throughput studies (**Figure 3**) [33].

PEG chains are universal linkers, as demonstrated by Darwish et al. In their work on phthalocyanine conjugated to a monoclonal antibody directed against multiple myeloma. They achieved good photodynamic activity in the nanomolar range [34]. Purushothaman et al. report their success in creating self-assembled nanoparticles of hydrophobic porphyrin and biotin with a PEG linker and encapsulated doxorubicin. They show synergistic effects of combining traditional chemotherapy and PDT (**Figure 4**) [35].

Such amphiphilic molecules can be more easily tested in aqueous solutions exhibiting different photophysical properties than in organic solvents. Mandal et al. demonstrated this in their publications on unsymmetrical porphyrins, chlorins and bacteriochlorins. The authors extensively study the photophysical properties of water-soluble compounds from these groups ready to be combined with other molecules or carriers [37–39]. Natural protoporphyrin IX can be very easily modified with PEG chains by esterification, which significantly increases its photodynamic activity. At the same time, pegylation substantially improves the therapeutic index, thus increasing the safety of use (**Figure 4**) [36]. The literature mentions the ability to form nanoparticles in the self-assembly of compounds with PEG chains in their structure without using additional stabilizers or surfactants [35, 40, 41]. In addition, the complexing properties of metals by these macrocycles make them excellent candidates for use in radio imaging with the help of radioactive isotopes such as copper ^{64}Cu [42]. The length and number of PEG chains have a significant influence on the properties of the final compounds. Mewis et al. noted that direct comparison is difficult because many authors publish compounds with a different number of substituents and largely non-uniform PEG chain length [43]. Kępczyński et al. Carried out studies of a hydrophobic porphyrin with one very long PEG chain with a mass of 8 kDa, including studies of aggregation, dissociation and interaction with liposomes. The authors note that adding PEGylated porphyrin increases the liposomes' size slightly while increasing their stability [44]. Later, in their work, a part of the same team compared the different porphyrin chain lengths and their in vitro efficacy. It is noteworthy that increasing the chain length increases the cytotoxicity in the light without affecting the

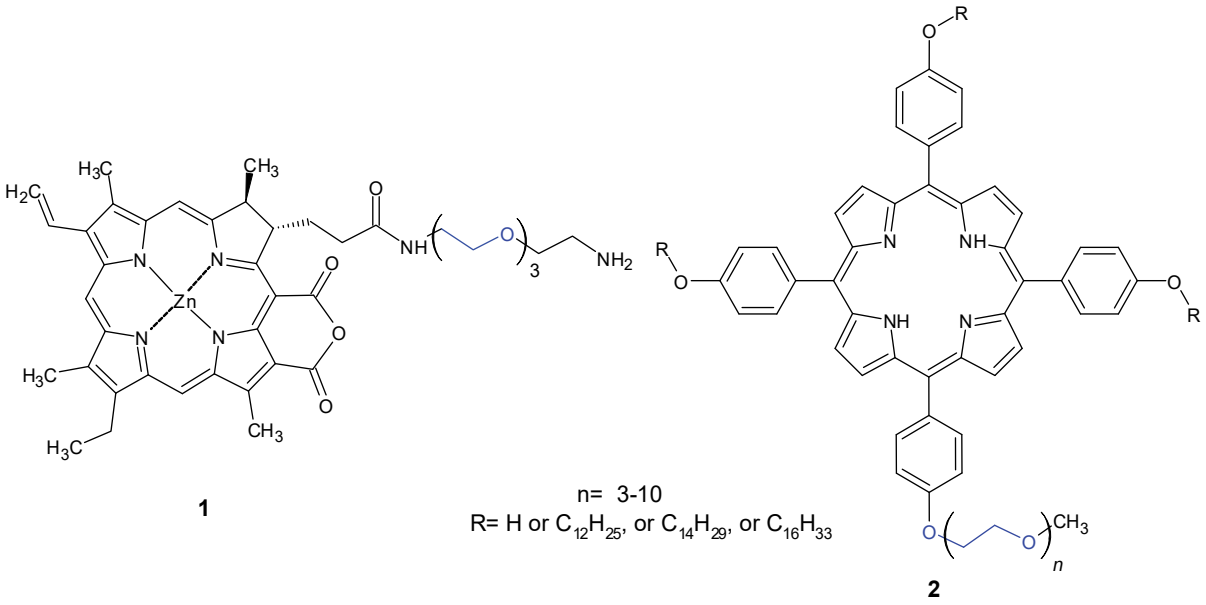

Figure 3. Purpurin 18 derivative designed by Pavlíčková et al. – 1 [32]and porphyrin derivatives – 2 designed by Zhdanova et al. [33]

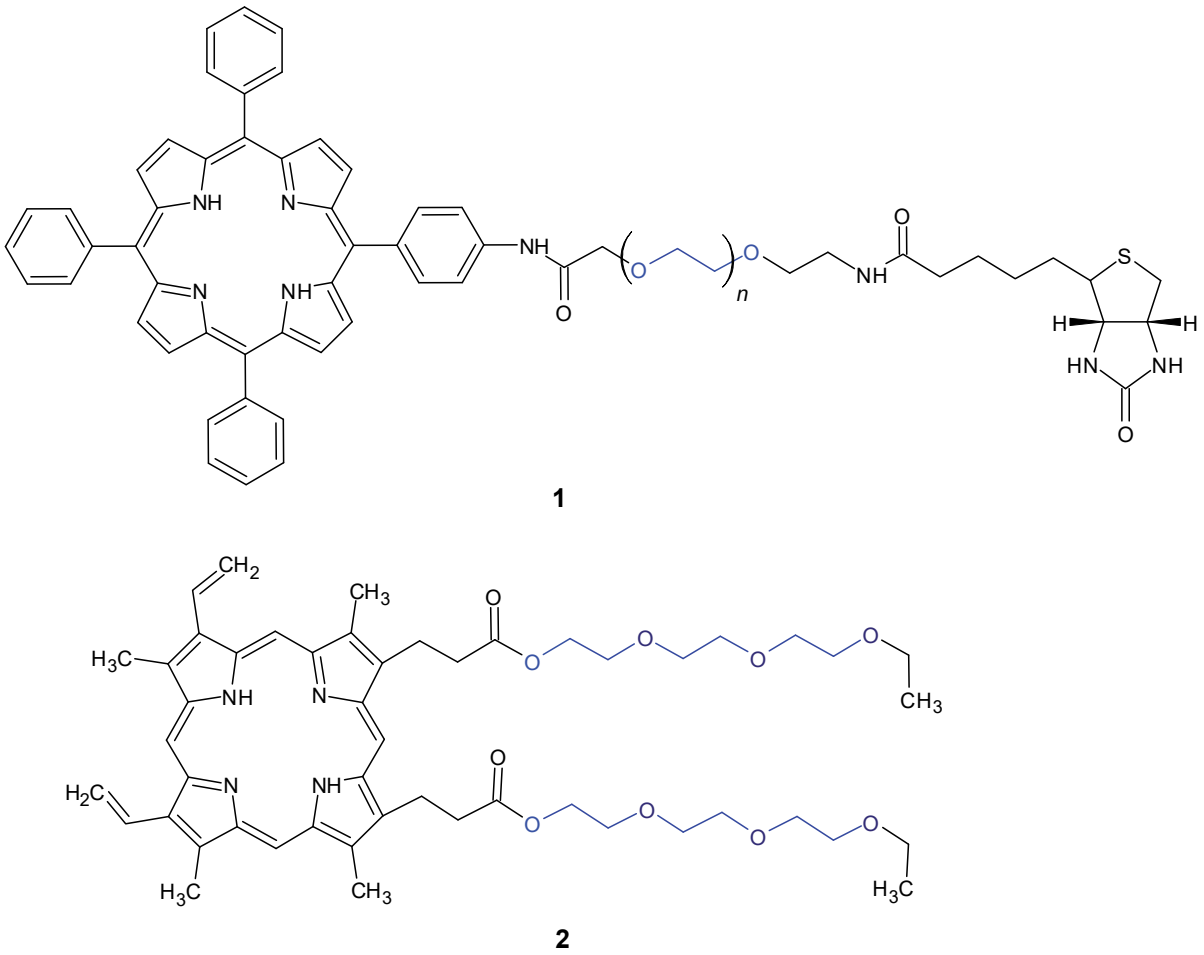

Figure 4. Porphyrin biotin conjugate with PEG linker – 1 [35], protoporphyrin IX PEG ester – 2 [36]

cytotoxicity in the dark. However, too long a chain reduces the efficacy of the compound. Therefore, PEG with a mass of approx. 2 kDa turned out to be the most optimal [45]. There is, however, a growing body of research in using short PEG chains below 1000Da, utilizing their small size to achieve similar results as their bigger brothers. Their main advantage is size uniformity which allows for much greater precision when tailoring the amphiphilic balance of the studied compound [46]. At the same time, it is worth noting that more is only sometimes better. For example, Sibrian-Vazquez et al. investigated the penetration of porphyrins modified with various amounts of polyethylene glycol chains inside the cell. They noticed that the presence of one and two chains in the molecule promotes the penetration of the test compound into the cells. Conversely, 3–4 chains significantly reduce their ability to penetrate the membranes [47]. On the other hand, in the literature, there are examples of numerous substituted phthalocyanines containing up to 8 short chains of PEG, which achieve excellent activity against cancer cells and viruses at the same time [48].

Classic therapy and small molecule drugs

The drugs most commonly used in traditional therapies are small molecules that target receptors or active sites in enzymes. Contrary to photodynamic therapy, traditional drugs must fit into small spaces present in macromolecules to modify their action. The placement of large functional groups, as PEG chains very often are, can have positive and negative consequences. Hamidi et al. reviewed several advantages, such as increased circulation time of PEG-modified drugs in the blood, alteration of the elimination profile towards biliary elimination instead of renal filtration, and accumulation in neoplastic cells [49].

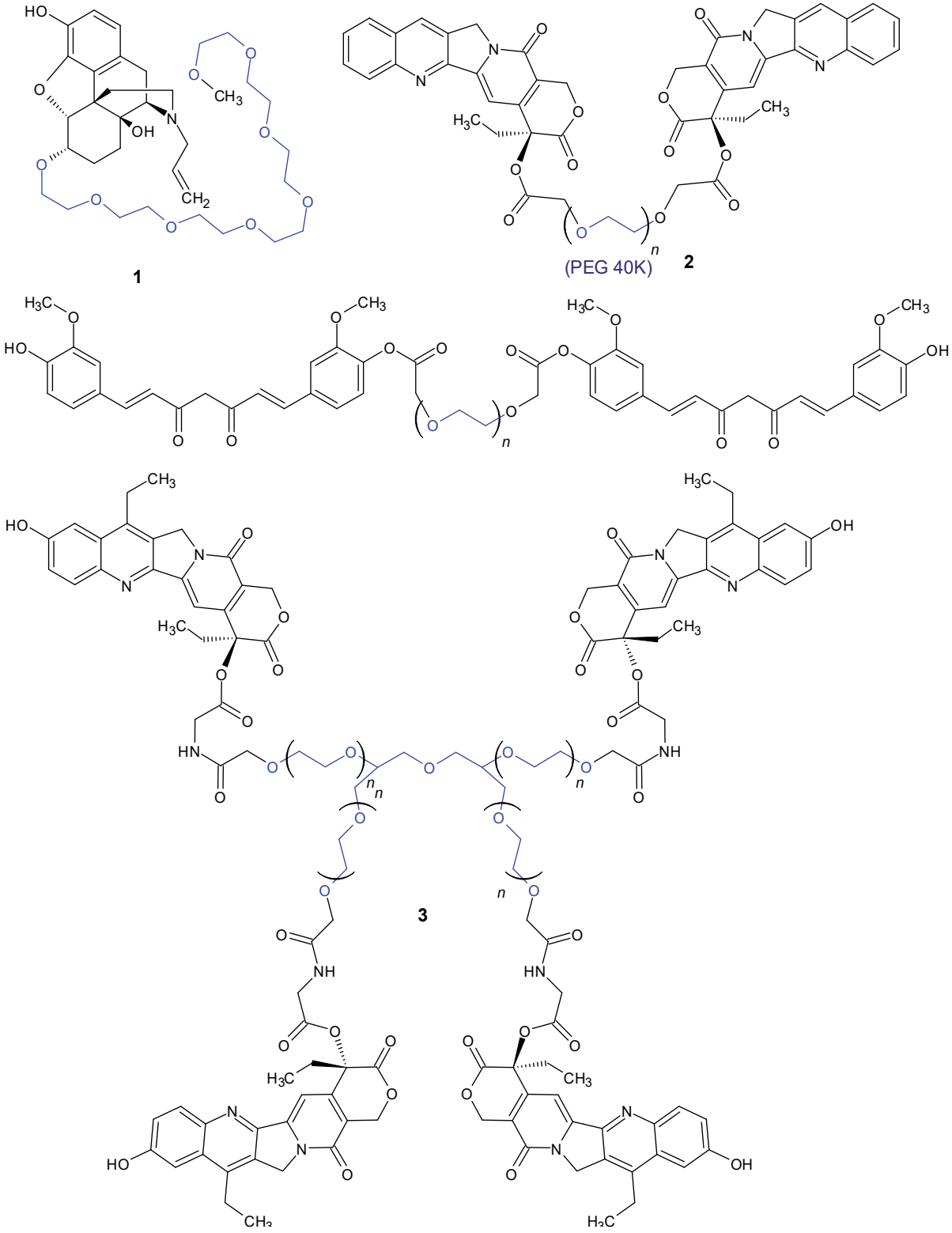

Figure 5. Structures of pegylated naloxol – 1 [54], pegylated campthotecin derivative – 2 [53] and campthotecin derivative with multibranched PEG – 3 [53]

An excellent example of the problems associated with small molecule pegylation is the research by Greenwald et al. Carried out in the 1990s. The taxol modifications they obtained became much more soluble in water, but unfortunately, they lost a significant part of their anticancer activity [50]. So far, most research has focused on the pegylation of large molecules such as proteins and enzymes. In modifying small molecule drug candidates, a few, including pegylated derivatives of irinotecan, camptothecin (**Figure 5**), doxorubicin and docetaxel, have proceeded to Phase III clinical trials [7, 51, 52]. Many other molecules, often PEG-modified cytostatic drugs in use today, are at an earlier stage. These include paclitaxel, carboplatin (**Figure 6**), gemcitabine, methotrexate and lamellarin derivatives (**Figure 6**). There are also reports on pegylated drugs from other therapeutic groups like e.g. acyclovir, gentamicin, zidovudine and amphotericin B [53]. In 2014, the EMA and the FDA approved a pegylated derivative of naloxol for treating constipation caused by opioids (**Figure 5**) [9].

An interesting pegylation variation is combining active particles with crown ethers (**Figure 6**). These cyclic versions of polyethene glycol can complex metal ions such as sodium and potassium. They can also lock small molecules of drugs inside them. Thus, they can significantly facilitate the penetration of the lipid membranes of cells. Their use for creating non-ionic liposomes by combining with a lipophilic molecule, e.g. cholesterol, has also been described [55].

Problems to be solved

Despite its very many desirable properties, the use of PEG is not free of drawbacks. In the last 20 years, reports have begun to emerge about the opposite of the expected effects of molecules in combination with PEG – shortening of circulation time, no reduction or even increased binding of drugs by proteins, and reduced ability to penetrate membranes. Studies of new combinations of therapeutics with PEG very often test only one type of PEG of a particular length or introduce a certain amount of PEG chains into the modified molecule without systematically comparing the effect of varying their amount on the properties of the resulting conjugate. Due to the production method, it is natural for the distribution of PEG molecular masses to span even often several thousand Da. The above contradicts the assumptions of bringing strictly formulated therapeutics with a defined structure to the market. The discovery of antibodies directed against PEG has become another problem. They pose a serious challenge because they can significantly affect the efficacy of all pegylated therapeutics admin-

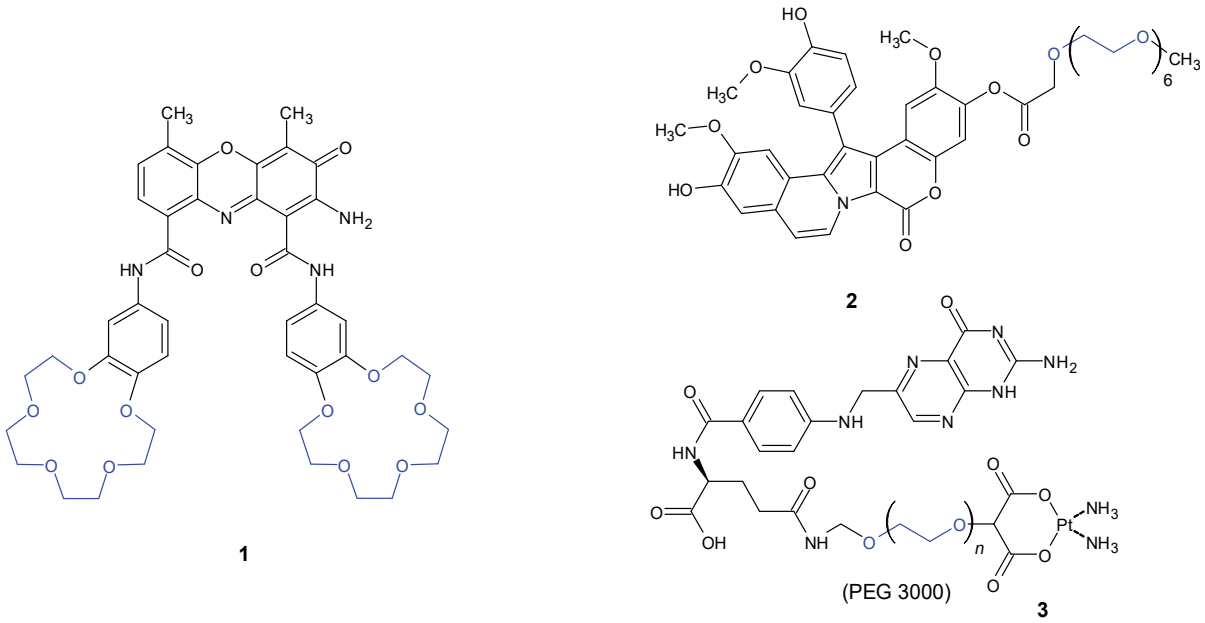

Figure 6. Antinocin conjugated with crown ethers – 1 [55], pegylated lamellarin derivative – 2 [53] and carboplatin with PEG linker and folate designed to target cancer cells – 3 [53]

istered subsequently, regardless of whether they are directed at treating different ailments [56, 57]. One of the challenges in using PEG is finding the appropriate balance in the amount. Adding too much PEG to the liposomes or copolymer nanoparticles results in too fast a release of the transported drug leading to a burst increase in concentration which can, of course, cause toxic effects. Depending on the modified particle and PEG chain chosen, liposomes and nanoparticles exhibit shorter circulation times and accumulate in the liver in the same way as non-pegylated versions. One possible explanation for this is the particle size increase after pegylation, making them easier to capture by RES (reticuloendothelial system) macrophages [56, 58–60]. A notable drawback of PEG is that while it is considered biocompatible, it is not biodegradable. Some pegylated drugs have been shown to accumulate in cytoplasmic vacuoles. Moreover, while currently, there are no known toxicological effects of this phenomenon, it still warrants further research. Especially as long-term consequences are completely unknown. An additional concern relates to the big size of PEG pendants in proteins and small molecules. While protecting them from elimination they can significantly lower their activity, by as much as 93% [61]. Most of this effect stems from the same shielding property of PEG as it can interfere with the therapeutic binding either to the active site where it is supposed to act or block the access of substrates to the active site of the pegylated enzyme [62]. Many of these concerns stem from no detailed research on the effects of the PEG chain length on the modified particle. The number of substitutions or the amount of PEG in a formulation is the second most important consideration.

Summary

Progress in medicine and pharmacy causes a constant increase in the number of therapeutics containing polyethylene glycol chains in their structure. An increasing representation of this compound type appears in the second and third clinical trial phases. Literature reports confirm that more and more new compounds are designed from scratch, taking into account the favourable properties of PEG. The emerging difficulties in the purification and response of the immune system are a reason to consider the direction of these modifications. Pegylation can also offer opportunities for the return to therapy of drugs that may have been discontinued due to their pharmacokinetic properties. It also improves the properties of currently used drugs that do not have an alternative in the form of newer drugs. Pegylation also opens up new possibilities for emerging compounds that might not proceed to the further research phase due to problems with formulation or bioavailability. In conclusion, pegylation is an excellent modification method, but more detailed studies are needed comparing the effects of different lengths and numbers of chains.

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Conflict of interest statement
The authors declare no conflict of interest.

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References

1. Greenwald RB, Choe YH, McGuire J, Conover CD. Effective drug delivery by PEGylated drug conjugates. Adv Drug Deliv Rev. 2003;55(2):217–50.
2. D'souza AA, Shegokar R. Polyethylene glycol (PEG): a versatile polymer for pharmaceutical applications. Expert Opin Drug Deliv [Internet]. 2016;13(9):1257–75. Available from: http://dx.doi.org/10.1080/17425247.2016.1182485
3. Herndon TM, Demko SG, Jiang X, He K, Gootenberg JE, Cohen MH, et al. U.S. Food and Drug Administration Approval: Peginterferon-alfa-2b for the Adjuvant Treatment of Patients with Melanoma. Oncologist [Internet]. 2012 Oct 1;17(10):1323–8. Available from: https://academic.oup.com/oncolo/article/17/10/1323/6400884
4. Jevševar S, Kunstelj M, Porekar VG. PEGylation of therapeutic proteins. Biotechnol J. 2010;5(1):113–28.
5. Vogel CL, Wojtukiewicz MZ, Carroll RR, Tjulandin SA, Barajas-Figueroa LJ, Wiens BL, et al. First and subsequent cycle use of pegfilgrastim prevents febrile neutropenia in patients with breast cancer: A multicenter, double-blind, placebo-controlled phase III study. J Clin Oncol. 2005;23(6):1178–84.
6. Dinndorf PA, Gootenberg J, Cohen MH, Keegan P, Pazdur R. FDA Drug Approval Summary: Pegaspargase (Oncaspar®) for the First-Line Treatment of Children with Acute Lymphoblastic Leukemia (ALL). Oncologist. 2007;12(8):991–8.
7. Mishra P, Nayak B, Dey RK. PEGylation in anti-cancer therapy: An overview. Asian J Pharm Sci [Internet]. 2016;11(3):337–48. Available from: http://dx.doi.org/10.1016/j.ajps.2015.08.011

8. FDA Approved PEGylated Drugs Up To 2022 | Biopharma PEG [Internet]. [cited 2022 Oct 18]. Available from: https://www.biochempeg.com/article/58.html
9. Park EJ, Choi J, Lee KC, Na DH. Emerging PEGylated non-biologic drugs. Expert Opin Emerg Drugs [Internet]. 2019;24(2):107–19. Available from: https://doi.org/10.1080/14728214.2019.1604684
10. Swierczewska M, Lee KC, Lee S. What is the future of PEGylated therapies? Expert Opin Emerg Drugs [Internet]. 2015;20(4):531–6. Available from: http://dx.doi.org/10.1517/14728214.2015.1113254
11. Yadav D, Dewangan HK. PEGYLATION: an important approach for novel drug delivery system. J Biomater Sci Polym Ed [Internet]. 2021;32(2):266–80. Available from: https://doi.org/10.1080/09205063.2020.1825304
12. Hoy SM. Pegcetacoplan: First Approval. Drugs [Internet]. 2021;81(12):1423–30. Available from: https://doi.org/10.1007/s40265-021-01560-8
13. Sravanthi S, Kumari MM, Sharma JVC, Peg R. A Critique View On Skytrofa. 2021;4(11):188–91.
14. Aschenbrenner DS. New Treatment for Polycythemia Vera. AJN, Am J Nurs [Internet]. 2022 Mar;122(3):18–9. Available from: https://journals.lww.com/10.1097/01.NAJ.0000822968.37066.5c
15. Jadach B. From the carrier of active substance to drug delivery systems. J Med Sci. 2017;86(3):231–6.
16. Ghosh S, Carter KA, Lovell JF. Liposomal formulations of photosensitizers. Biomaterials [Internet]. 2019;218(April):119341. Available from: https://doi.org/10.1016/j.biomaterials.2019.119341
17. Allen TM. Long-circulating (sterically stabilized) liposomes for targeted drug delivery. Trends Pharmacol Sci. 1994;15(7):215–20.
18. Nawalany K, Rusin A, Kepczyński M, Mikhailov A, Kramer-Marek G, Śnietura M, et al. Comparison of photodynamic efficacy of tetraarylporphyrin pegylated or encapsulated in liposomes: In vitro studies. J Photochem Photobiol B Biol. 2009;97(1):8–17.
19. Duggan ST, Keating GM. Pegylated Liposomal Doxorubicin. Drugs [Internet]. 2011 Dec;71(18):2531–58. Available from: http://link.springer.com/10.2165/11207510-000000000-00000
20. Knudsen NØ, Rønholt S, Salte RD, Jorgensen L, Thormann T, Basse LH, et al. Calcipotriol delivery into the skin with PEGylated liposomes. Eur J Pharm Biopharm. 2012;81(3):532–9.
21. Wang X, Song Y, Su Y, Tian Q, Li B, Quan J, et al. Are PEGylated liposomes better than conventional liposomes? A special case for vincristine. Drug Deliv [Internet]. 2016;23(4):1092–100. Available from: http://dx.doi.org/10.3109/10717544.2015.1027015
22. Amoozgar Z, Yeo Y. Recent advances in stealth coating of nanoparticle drug delivery systems. Wiley Interdiscip Rev Nanomedicine Nanobiotechnology. 2012;4(2):219–33.
23. Jokerst J V., Lobovkina T, Zare RN, Gambhir SS. Nanoparticle PEGylation for imaging and therapy. Nanomedicine. 2011;6(4):715–28.
24. Locatelli E, Franchini MC. Biodegradable PLGA-b-PEG polymeric nanoparticles: Synthesis, properties, and nanomedical applications as drug delivery system. J Nanoparticle Res. 2012;14(12):1–17.
25. Zhu Y, Fang Y, Borchardt L, Kaskel S. PEGylated hollow mesoporous silica nanoparticles as potential drug delivery vehicles. Microporous Mesoporous Mater [Internet]. 2011;141(1–3):199–206. Available from: http://dx.doi.org/10.1016/j.micromeso.2010.11.013
26. Kübler AC. Photodynamic therapy. Med Laser Appl. 2005;20(1):37–45.
27. MACDONALD IJ, DOUGHERTY TJ. Basic principles of photodynamic therapy. J Porphyrins Phthalocyanines [Internet]. 2001 Feb;05(02):105–29. Available from: http://www.worldscientific.com/doi/abs/10.1002/jpp.328
28. Baskaran R, Lee J, Yang SG. Clinical development of photodynamic agents and therapeutic applications. Biomater Res. 2018;22:1–8.
29. Luciano M, Bruckner C. Modifications of porphyrins and hydroporphyrins for their solubilization in aqueous media. Vol. 22, Molecules. 2017.
30. Milton Harris J, Martin NE, Modi M. Pegylation: A novel process for modifying pharmacokinetics. Clin Pharmacokinet. 2001;40(7):539–51.
31. Gunaydin G, Gedik ME, Ayan S. Photodynamic Therapy for the Treatment and Diagnosis of Cancer–A Review of the Current Clinical Status. Front Chem. 2021;9(August):1–26.
32. Pavlíčková V, Rimpelová S, Jurášek M, Záruba K, Fähnrich J, Křížová I, et al. PEGylated purpurin 18 with improved solubility: Potent compounds for photodynamic therapy of cancer. Molecules. 2019;24(24):1–25.
33. Zhdanova KA, Cherepanova KS, Bragina NA, Mironov AF. New pegylated unsymmetrical meso-arylporphyrins as potential photosensitizers. Macroheterocycles. 2016;9(2):169–74.
34. Darwish WM, Bayoumi NA, El-Shershaby HM, Allahloubi NM. Targeted photoimmunotherapy based on photosensitizer-antibody conjugates for multiple myeloma treatment. J Photochem Photobiol B Biol [Internet]. 2020;203(January):111777. Available from: https://doi.org/10.1016/j.jphotobiol.2020.111777
35. Purushothaman B, Choi J, Park S, Lee J, Samson AAS, Hong S, et al. Biotin-conjugated PEGylated porphyrin self-assembled nanoparticles co-targeting mitochondria and lysosomes for advanced chemo-photodynamic combination therapy. J Mater Chem B. 2019;7(1):65–79.
36. Wierzchowski M, Łażewski D, Tardowski T, Grochocka M, Czajkowski R, Sobiak S, et al. Nanomolar photodynamic activity of porphyrins bearing 1,4,7-trioxanonyl and 2-methyl-5-nitroimidazole moieties against cancer cells. J Photochem Photobiol B Biol [Internet]. 2020;202(October 2019):111703. Available from: https://doi.org/10.1016/j.jphotobiol.2019.111703
37. Mandal AK, Sahin T, Liu M, Lindsey JS, Bocian DF, Holten D. Photophysical comparisons of PEGylated porphyrins, chlorins and bacteriochlorins in water. New J Chem [Internet]. 2016;40(11):9648–56. Available from: http://dx.doi.org/10.1039/C6NJ02091G
38. Zhang N, Jiang J, Liu M, Taniguchi M, Mandal AK, Evans-Storms RB, et al. Bioconjugatable, PEGylated

hydroporphyrins for photochemistry and photomedicine. Narrow-band, near-infrared-emitting bacteriochlorins. New J Chem [Internet]. 2016;40(9):7750–67. Available from: http://dx.doi.org/10.1039/C6NJ01155A
39. Liu M, Chen CY, Mandal AK, Chandrashaker V, Evans-Storms RB, Pitner JB, et al. Bioconjugatable, PEGylated hydroporphyrins for photochemistry and photomedicine. Narrow-band, red-emitting chlorins. New J Chem [Internet]. 2016;40(9):7721–40. Available from: http://dx.doi.org/10.1039/C6NJ01154C
40. Ding F, Li C, Xu Y, Li J, Li H, Yang G, et al. PEGylation Regulates Self-Assembled Small-Molecule Dye–Based Probes from Single Molecule to Nanoparticle Size for Multifunctional NIR-II Bioimaging. Adv Healthc Mater. 2018;7(23):1–9.
41. Hou W, Xia F, Alves CS, Qian X, Yang Y, Cui D. MMP2-Targeting and Redox-Responsive PEGylated Chlorin e6 Nanoparticles for Cancer Near-Infrared Imaging and Photodynamic Therapy. ACS Appl Mater Interfaces. 2016;8(2):1447–57.
42. Cheng L, Jiang D, Kamkaew A, Valdovinos HF, Im HJ, Feng L, et al. Renal-Clearable PEGylated Porphyrin Nanoparticles for Image-Guided Photodynamic Cancer Therapy. Adv Funct Mater. 2017;27(34):1–10.
43. Mewis RE, Savoie H, Archibald SJ, Boyle RW. Synthesis and phototoxicity of polyethylene glycol (PEG) substituted metal-free and metallo-porphyrins: Effect of PEG chain length, coordinated metal, and axial ligand. Photodiagnosis Photodyn Ther. 2009;6(3–4):200–6.
44. Kepczyński M, Nawalany K, Jachimska B, Romek M, Nowakowska M. Pegylated tetraarylporphyrin entrapped in liposomal membranes. A possible novel drug-carrier system for photodynamic therapy. Colloids Surfaces B Biointerfaces. 2006;49(1):22–30.
45. Nawalany K, Rusin A, Kepczynski M, Filipczak P, Kumorek M, Kozik B, et al. Novel nanostructural photosensitizers for photodynamic therapy: In vitro studies. Int J Pharm [Internet]. 2012;430(1–2):129–40. Available from: http://dx.doi.org/10.1016/j.ijpharm.2012.04.016
46. Lazewski D, Kucinska M, Potapskiy E, Kuzminska J, Tezyk A, Popenda L, et al. Novel Short PEG Chain-Substituted Porphyrins: Synthesis, Photochemistry, and In Vitro Photodynamic Activity against Cancer Cells. Int J Mol Sci. 2022;23(17).
47. Sibrian-Vazquez M, Jensen TJ, Vicente MGH. Synthesis and cellular studies of PEG-functionalized meso-tetraphenylporphyrins. J Photochem Photobiol B Biol. 2007;86(1):9–21.
48. Sobotta L, Wierzchowski M, Mierzwicki M, Gdaniec Z, Mielcarek J, Persoons L, et al. Photochemical studies and nanomolar photodynamic activities of phthalocyanines functionalized with 1,4,7-trioxanonyl moieties at their non-peripheral positions. J Inorg Biochem [Internet]. 2016;155:76–81. Available from: http://www.sciencedirect.com/science/article/pii/S0162013415301112
49. Hamidi M, Azadi A, Rafiei P. Pharmacokinetic consequences of pegylation. Drug Deliv. 2006;13(6):399–409.
50. Greenwald RB, Pendri A, Bolikal D. Highly Water Soluble Taxol Derivatives: 7-Polyethylene Glycol Carbamates and Carbonates. J Org Chem [Internet]. 1995 Jan 1;60(2):331–6. Available from: https://pubs.acs.org/doi/abs/10.1021/jo00107a010
51. Parveen S, Arjmand F, Tabassum S. Clinical developments of antitumor polymer therapeutics. RSC Adv. 2019;9(43):24699–721.
52. Zhang X, Wang H, Ma Z, Wu B. Effects of pharmaceutical PEGylation on drug metabolism and its clinical concerns. Expert Opin Drug Metab Toxicol. 2014;10(12):1691–702.
53. Li W, Zhan P, De Clercq E, Lou H, Liu X. Current drug research on PEGylation with small molecular agents. Prog Polym Sci [Internet]. 2013;38(3–4):421–44. Available from: http://dx.doi.org/10.1016/j.progpolymsci.2012.07.006
54. Floettmann E, Bui K, Sostek M, Payza K, Eldon M. Pharmacologic profile of naloxegol, a peripherally acting μ-opioid receptor antagonist, for the treatment of opioid-induced constipation. J Pharmacol Exp Ther. 2017;361(2):280–91.
55. Chehardoli G, Bahmani A. The role of crown ethers in drug delivery. Supramol Chem [Internet]. 2019;31(4):221–38. Available from: https://doi.org/10.1080/10610278.2019.1568432
56. Verhoef JJF, Anchordoquy TJ. Questioning the use of PEGylation for drug delivery. Drug Deliv Transl Res. 2013;3(6):499–503.
57. Armstrong JK, Hempel G, Koling S, Chan LS, Fisher T, Meiselman HJ, et al. Antibody against poly(ethylene glycol) adversely affects PEG-asparaginase therapy in acute lymphoblastic leukemia patients. Cancer [Internet]. 2007 Jul 1;110(1):103–11. Available from: https://onlinelibrary.wiley.com/doi/10.1002/cncr.22739
58. Sebak AA. Limitations of pegylated nanocarriers: Unfavourable physicochemical properties, biodistribution patterns and cellular and subcellular fates. Int J Appl Pharm. 2018;10(5):6–12.
59. Xu J, Gattacceca F, Amiji M. Biodistribution and Pharmacokinetics of EGFR-Targeted Thiolated Gelatin Nanoparticles Following Systemic Administration in Pancreatic Tumor-Bearing Mice. Mol Pharm [Internet]. 2013 May 6;10(5):2031–44. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3624763/pdf/nihms412728.pdf
60. Rafiei Pedram HA. IJN-121881-docetaxel-loaded-plga-and-plga-peg-nanoparticles-for-intrave. Int J Nanomedicine. 2017;12:935–47.
61. Zalipsky S, Harris JM. Introduction to Chemistry and Biological Applications of Poly(ethylene glycol). Vol. 680, ACS Symposium Series. 1997. 1–13 p.
62. Zhang F, Liu MR, Wan HT. Discussion about several potential drawbacks of PEGylated therapeutic proteins. Biol Pharm Bull. 2014;37(3):335–9.