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Recent developments in phosphorus-containing ester prodrugs and its prospectives

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Abstract. Prodrugs has been established as a strategy to improve the medical properties of their parent drugs, and thereby enhance the possibility of these potential drugs to cure the disease but this modified drugs should be cautious as their properties like issue distribution, efficacy and toxicity may change. In the current developed modifying strategies, esterification is among the most common ones, which masks charged hydrophilic groups with ester promoieties so that permeation ability of drugs through the membrane will enhance. Among various esters, phosphorus-containing ester prodrugs are attractive due to close relationship between atom phosphorus and key bioactive compounds including DNA or RNA, phospholipid, proteins. Herein, we will review the developments of these prodrugs, especially phosphate and phosphonate prodrugs, their synthetic methods, pros and cons, application and methods for characterization, with which specific examples or clinical trials will be attached for clearer description Ultimately, we prospect that the phosphorus-containing prodrugs will for certain be further studied and improved and be an indispensable role in medical cares.

Keywords: ester prodrugs, phosphorus

1. Introduction

The utilization of modern drug technology such as high-throughput screening[1]-[2] and combinatorial chemistry[3][4], helped the discovery of novel molecules with high pharmacological potency, but this disguised boon is not so satisfactory as these drug candidates may be problematic in some of their physicochemical properties, which regulates the drug development.[5]

Prodrugs – chemically modified bioactive agents which release the active drugs after transformation *in vivo*, has been established as a strategy to improve the physicochemical, pharmacokinetic properties of their parent drugs, and thereby enhance the possibility of these potential drugs to cure the disease. These strengthened capabilities include drug solubility; pharmaceutical stability; biological activity; drug targeting, etc. Meanwhile, a suitable modification should be taken serious and complete consideration as the altered functional groups of these compounds may change the tissue distribution, efficacy and toxicity of the parent drugs.[6]

Ester prodrug is one of the most common prodrugs in development. With some charged hydrophilic groups, such as carboxylic acids or hydroxyl groups, masked by the ester promoieties, the lipophilicity of the drugs increase, which enable their permeation through the membrane. The efficacy of the facilely accessible ester prodrugs can also be guaranteed as ester bonds will be easily hydrolyzed by various and ubiquitous esterases in blood, liver and other organs. [6]Until now, a wide range of ester prodrugs has been developed, like carbonates, sulfates, among which the phosphorus-containing esters are worth noticing. Being key to all life processes, atom phosphorus exists in almost every compounds which our bodies consist of, involving DNA or RNA, phospholipid, proteins, which suggests the privileged roll of phosphate in the ester prodrugs.[7]

In this article, we will review some of the developments in the phosphorus-containing ester prodrugs, including the synthesis of diverse phosphate esters prodrugs, the pros and cons of these prodrugs, the application of these compounds, the methods to characterize their properties and, finally, summarize them and make comments on the prospect of these beneficial molecules.

2. Synthesis

To modify the pharmacological potent compounds for their desired effects, various chemical methods have been developed to synthesize the phosphorylated derivatives. The following will cover three types of phosphorylated prodrugs synthesis: classical phosphorylation; phosphoramidate prodrugs and phosphorylation of polysaccharides.

2.1. Classical phosphorylation

One of the most effective routes for phosphorylation is Atherton–Todd reaction. [8]In this reaction, the P-H bond of dialkyl phosphonates is oxidated into a highly reactive P-Cl bond by using base and CCl4, then the prodrugs can be accessible after the parent drugs, acting as a nucleophile, interact with the dialkyl chlorophosphates.(Figure 1) Despite of the mild reaction conditions and high efficacy, the toxicity of CCl4 dramatically restricts its utilization. This inspires the creation of novel halogenation reagents and direct cross coupling methods, which reduces its potential risks to human health and environment and improves its versatility.

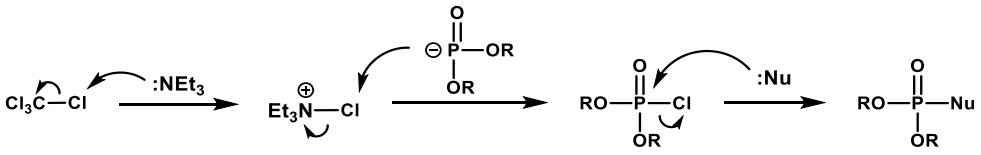

Figure 1. Purposed Mechanism of Atherton–Todd Reaction

Another historically known reaction for the generation of the P-C bonds is Michaelis–Arbuzov reaction. [9]The first step in classical Michaelis–Arbuzov reaction involves the attack of the lone pair of electrons from the phosphor atoms of the phosphite to the alkyl halides, producing an unstable quasiphosphonium intermediate. Then, the left halides anion attacks the alkyl group and form the phosphonate esters.(Figure 2) However, the efficiency of this pathway can not be ensured especially when there exists multiple reactive functional groups which hinder the selectivity and product purity.

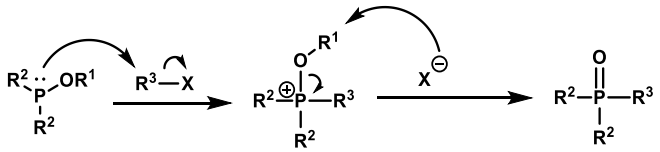

Figure 2. Purposed Mechanism of Michaelis–Arbuzov Reaction

Perkow reaction provide a route to synthesize enol phosphates. Similar to the MA reaction, the reaction starts with phosphites reacting with α-halo carbonyl compounds to afford cationic species. [10]The cation then undergoes a second nucleophilic substitution reaction to form a dealkylated salt, while the halide anion attacks one of the phosphite alkoxy groups to generate an enol phosphate.(Figure 3)

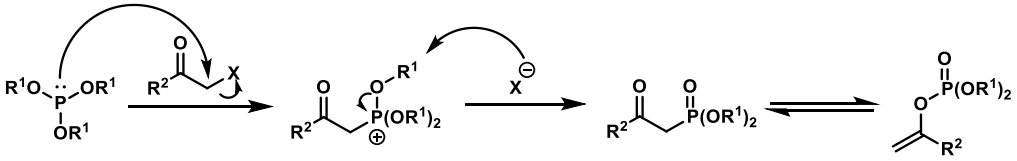

Figure 3. Purposed Mechanism of Perkow Reaction

2.2. Phosphoramidate prodrugs

The ProTide – a prodrug form of a nucleotide – consist of a mixed ester and phosphoryl amidate, which have been an extremely efficient prodrug strategy since its first launch by Christopher McGuigan.[11] In this approach, oxygen atoms of the phosphate groups in the nucleoside monophosphate analogues will be masked to keep them neutral at physiological pH and increase their permeation through the cell membrane. Inside the cells, an enzymatical cleavage will take place to release the efficacious molecules.

The classical preparation method is to introduce amines and alcohols stepwise into phosphorus dichloride by a strong base such as tBuMgCl or N-methylimidazole, which will not only neutralize the acidic species to keep the reaction progress, but also promote the reaction by activating the phosphoramide intermediate and accelerating the esterification (Figure 4). [12] This reaction

is superior in its selectivity and purification, but may be incompatible with some sensitive substrates due to complex conditions and difficulties in controlling regioselectivity.

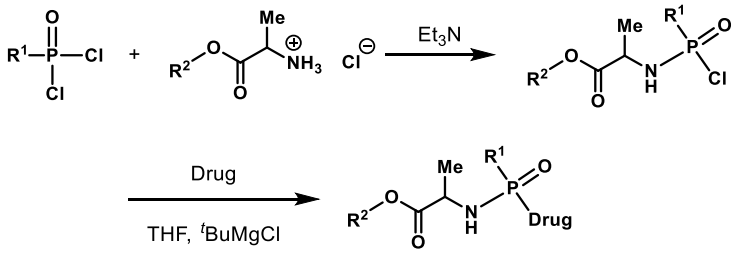

Figure 5. Preparation of aminophosphate prodrugs by classical preparation method

For preparation of alcohol-aryl-aminophosphate prodrugs, a transesterification strategy was developed. [13]Utilizing *N*-diphenylphosphoryl amino acid esters as the phosphoryl donor, this DBU-catalyzed protocol successfully realizes the phosphorylation of a range of primary or secondary alcohols under mild and concise conditions, enabling the synthesis of a variety of prodrug analogues of nucleosides containing nucleophilic functional groups. (Figure 5) Meanwhile, various amino acid esters are also compatible with this practical route.

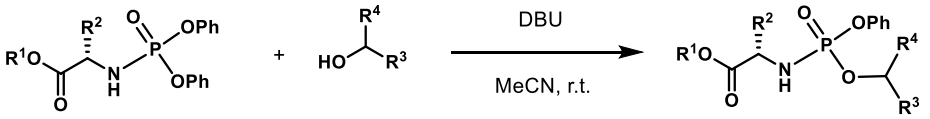

Figure 6. Preparation of alcohol-aryl-aminophosphate prodrugs by DBU catalysis

The phosphorylated phenolic pharmaceuticals can be reached by the similar transesterification strategy.(Figure 6) [14]Despite the unprotected and problematic nucleophilic groups, this pathway shows highly selective phosphorylation of phenolic hydroxyl groups. In addition, phenol cleavage from the phosphoryl group donor can be fine-tuned by agjusting the amino acid residues.

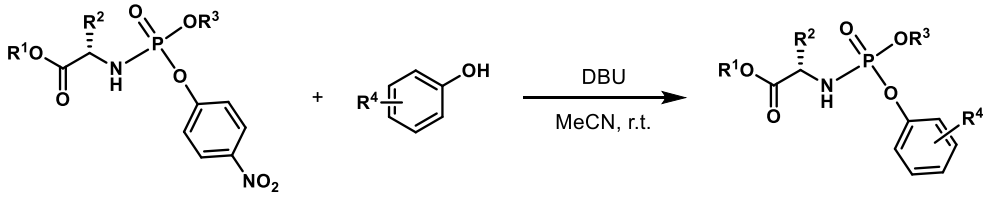

Figure 7. Preparation of phenolic aminophosphate prodrugs by DBU catalysis

2.3. Phosphorylated polysaccharides

Polysaccharides are a great group of vital and bioactive molecules present in our body. Commonly, without prolix protection strategies, phosphorylation of these compounds are problematic due to the multiple hydroxyl groups existing in them. Though there actually are some feasible and convenient methods to realize the phosphorylation process, by using POCl3[15], sodium phosphate[16], phosphoric acid and anhydride[17] or phosphorus pentoxide[18], none of them succeed in completing the reaction with both high selectivity and high replacement levels and, even worse, the polysaccharides risks degradation or decomposition under harsh conditions such as high temperatures or strong acids.(Figure 8)

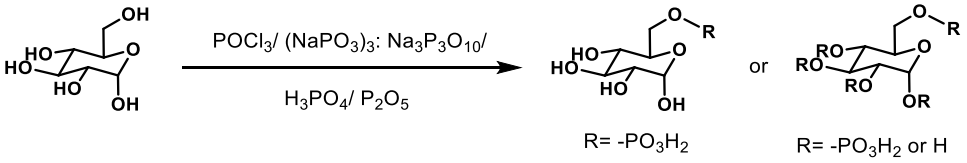

Figure 9. Methods for preparation of phosphorylated polysaccharides

3. Pros and cons of the P-containing ester prodrugs

When modified into organophosphorus chemicals, both significant and minor differences will appear in properties of the parent drugs. While some of them will be promising, others may instead impede with their efficacy or even bring danger. The following part will then describe how the phosphorylation is not an unalloyed boon.

3.1. Pros

For the hydrophobic drugs, neither might it solved appropriately in the blood to be delivered smoothly, nor it can easily come through the epithelial membrane which is a fat-abundant environment. However, equipped with the phosphate group, the prodrugs can be soluble in our blood because of the additional hydroxyl groups. The permeability also increases especially when another long-chain alkyl group is attached to the phosphate, which vividly mimic the structure of the phospholipid.[20] For example, flavopyriol, a potent CDK-9 inhibitor, is highly permeable but has pH-dependent solubility: 0.02 mg/ml in physiological pH, which is why formulation of its oral dosage forms get hamstringed. Therefore, its phosphate prodrugs are synthesized to overcome this challenge. It has been reported that the phosphorylated prodrug demonstrated enhanced solubility: 9.5 mg/ml under neutral condition, hundreds times of that of the parent drugs.[19]

Phosphate prodrugs are acknowledged for their stability, prolonged half-life, and capacity to cleavage the parent drug and phosphate groups via phosphatase-catalyzed bioconversion in vivo. Furthermore, in the physicological pH range of 7.0-7.4, the phosphate groups will deprotonate irreversibly and carry negative charges, which will repulse other nucleophilic or negatively charged chemicals and stop the non-enzymatic cleavage, and therefore prolong the half-life of these prodrugs. For example, the triptolide phosphonooxymethyl prodrug could stay unreactive at neutral pH, with an estimated half-life of approximately 2 years at 4 °C, and hardly interacts with the alkaline phosphatase.[21]

The structure of a compound will make a decisive influence on its pharmacological potency, which is why phosphorylation modification can alter the parent drugs' biological activities. For instance, The activity of a phosphoesterification modification of icariin in promoting osteogenic differentiation of MC3T3-E1 Subclone 14 can be higher than that of icariin itself. Another phosphorylated molecule in this report also depicts an enhanced antitumor capability compared to the raw one.[22]

Tumor cells often have higher levels of specific enzymes compared to the normal tissue cells. With delicate phosphorylation on the molecules, we can realize drug targeting refinement as, only with these unique proteins or enzymes, the prodrugs could be broken down and release the active antitumor ingredients. Elevated concentrations of these chemicals can effectively inhibit the growth of tumor, with relatively minor interaction with healthy cells..[22] A prominent example is the HepDirect prodrug for liver disease. They are highly sensitive to oxidation mediated by cytochrome P450 family 3 subfamily A member 4 (CYP3A4), which is excessively expressed in hepatocytes but relatively absent in other organs, so enhanced targeting and specificity and reduced side effects can be reached with this technique.[23]

3.2. Cons

However, potential cytotoxicity of the widely used P-containing moieties is not negligible as the aryl moiety liberated from the prodrugs can cause off-target toxicity.[24] Study showed greater and more rapid toxicity on primary cultured mouse astrocytes than targeted tumour cell lines.

Chronic kidney disease-mineral and bone disorder (CKD-MBD), a recently acknowledged metabolic abnormality, features impaired circulation of calcium, phosphorus, para-thyroid hormone, and vitamin D, which would lead to a series of syndromes including hyperphosphatemia.[25] Since the patients usually consume a variety of oral medications, the phosphorus intake will also shoot up. For the P-containing prodrugs, approximately 60% is absorbed in the intestine, which will be an extra burden on the patients as their renal clearance of phosphorus by HD is limited.

4. Applications

Nowadays, the P-containing prodrugs have been an inseparable role in the fight of diseases of all aspects. The following would introduce several representative prodrugs evaluated in the clinical trials.

4.1. Phosphate prodrugs

4.1.1. Lufotrelvir

Lufotrelvir (PF-07304814), developed and studied by Pfizer, Inc., is a remedy for COVID-19.(Figure 8) It will metabolized in vivo to its parent pharmacoactive compound, PF-00835231. Until now, there are three phase 1 clinical studies and two phase 3

studies reported. In the result-posted studies, phase 1 study NCT04535167 displays the safety, tolerability, and pharmacokinetics of lufotrelvir, in patients hospitalized with SARS-CoV-2 virus infection, while the phase 3 study NCT05780541 illustrates the safety and effectiveness of lufotrelvir in treating COVID-19 infected people.

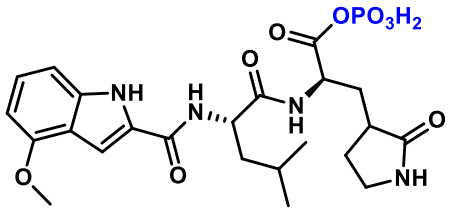

Figure 10. Lufotrelvir

4.1.2. Psilocybin

Psilocybin is a naturally occurring alkaloid with potential to eliminate depression and other mental disorders.(Figure 9) This prodrug will be metabolized to psilocin in *in vivo* system. In the ongoing phase 1 study, the physiological and psychological effects of psilocybin and psilocin taken orally by pill is compared in healthy adults. Also, various studies, like phase 1 study NCT03300947 and phase 2 study NCT05312151, its safety, tolerability and other applications in mental diseases are still under estimation.

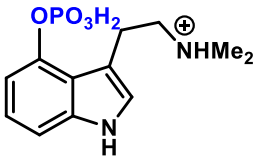

Figure 11. Psilocybin

4.2. Phosphonate prodrugs

4.2.1. Tenofovir exalidex

Tenofovir Exalidex (CMX157) is a lipid conjugate of the acyclic nucleotide analog Tenofovir with activity against both wild-type and antiretroviral drug-resistant HIV strains, including multidrug nucleoside/nucleotide analog-resistant viruses.(Figure 10) Bearing long ()carbon chain and masking its one anion of the phosphonate, its efficacy against HIV gets improved. In clinical trials, phase 1 study NCT01080820 evaluate the safety, tolerability and pharmacokinetic study of a single dose of CMX157 in Healthy Volunteers while phase 1 study NCT03284164 evaluated the side effect--renal impairment on the PK of Tenofovir Exalidex.

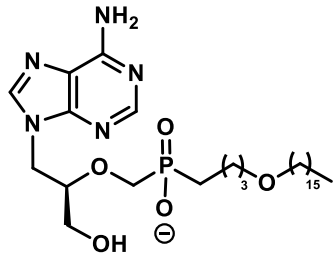

Figure 12. Tenofovir Exalidex

4.2.2. Managlinat dialanetil

Managlinat dialanetil is a bis-alanine ethyl ester-protected phosphonate which is applicable to inhibit fructose 1,6-bisphosphatase.(Figure 11) It was shown to have a tenfold higher anti-type-2-diabetes capability than the free phosphonic acid. The phase 2 trial NCT00290940 evaluated the glucose lowering effect, safety and tolerability of this prodrug, but it was ultimately withdrawn due to insufficient ability to counter long-term glucose exposure.

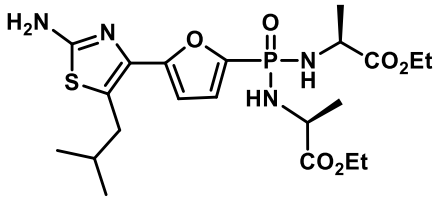

Figure 13. Managlinat dialanetil

4.3. Mixed amidate/esters

4.3.1. Tenofovir alafenamide

Tenofovir alafenamide is novel nucleoside reverse transcriptase inhibitor (NRTI) developed for the treatment of chronic hepatitis B in adults.(Figure 12) It is seen as an upgraded version of the marketed drug Tenofovir Disoproxil Fumarate. As it has been shown to have a very dramatic higher antiviral potency and better safety, especially in improvement of renal function and skeletal safety parameters. As a successful commercial prodrug, it has been evaluated and investigated in abundant clinical trials. In Phase 1 study NCT05423106, its safety and tolerability was scoped including single ascending dose (SAD) and multiple ascending dose (MAD) administration to both healthy adult participants and chronic hepatitis B (CHB) participants. Another Phase 1 study NCT02984852 evaluated the relative bioavailability of fixed-dose combination tablet Darunavir/Cobicistat/Emtricitabine/ Tenofovir Alafenamide (D/C/F/TAF) as a whole tablet, as a split tablet, and as crushed tablet in healthy adults. In Phase 2 clinical trials NCT05423834 and NCT03115736, its effects on kidneys of chronic hepatitis B patients and HIV/HBV-coinfected participants were respectively described.

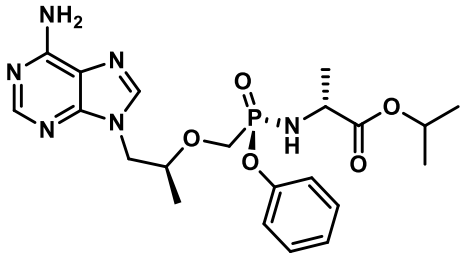

Figure 14. Tenofovir alafenamide

4.3.2. Sofosbuvir

Sofosbuvir is in the nucleotide analog family of medications.(Figure 13) It can cure hepatitis C infectors by blocking the hepatitis C NS5B protein with combination of other anti-HCV drugs. Being an approved prodrug, its properties has been solidly and detailly estimated. In Phase 1 clinical trial NCT01441180, its safety and effects was tested to see if it can work alone or with other drugs. Phase 2 trial NCT02165189 depicts its efficacy and safety with and without Ribavirin in participants with recurrent genotype 1 Hepatitis C post-orthotopic liver transplant.

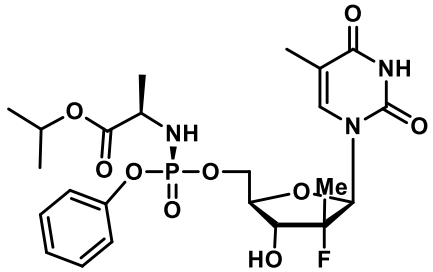

Figure 15. Sofosbuvir

4.4. Summary of functional groups

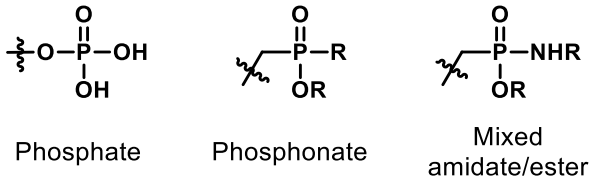

Figure 16. Common Functional Groups in Phosphorus Prodrugs

Compared with the original drugs, the phosphate-modified ones were equipped with better solubility and bioactivity as the polar phosphate groups attract the water and promote solvation. Meanwhile, since this bonding is resistant to nonenzymatic hydrolysis, its stability also increased. Then, with one of the hydroxyl groups replaced with carbon groups, especially the long carbon chains, the ionic part in this molecule get masked so that its lipophilicity get improved. And the mixed substitution pattern introduces a stereogenic center at the phosphorus atom, which further tunes its targeting capacity while solving problems such as poor cellular uptake, poor conversion to the active component or to release less toxic byproducts.

5. Analytical strategies

To gain a full insight on the druggable molecules and predict its potential in the medical applications, pharmacologists have included a variety of analytical techniques to explore the properties of the drugs.

5.1. Physical properties

The physical properties plays an unplaceable role in the kinetic performance of the drugs. Solubility is one of decisive and key parameters affecting the drug efficacy as the solubility insufficiencies in water will hinder its oral activities while weakness in lipophilicity will hurt its absorption and penetration, which finally obstruct its formulation. A common method for the measurement of aqueous solubility is miniaturized shake flask method, but large amount of sample drugs may be required in some protocols. In 2015, Bharate has reported a a simplified, fast and reliable 96-well plate method whose solubility obtained was well in line with literature reported values.[26]

The chemical structure should be in the sight of the analysis of the prodrugs so that we can ensure its purity and gain deeper insight into their properties. Fourier Transform Infrared Spectroscopy (FT-IR) is certain dominant technology in the field of structure analysis due to its intermediate measurement, outstanding resolution and minute sample requirements. For the phosphorylated compounds, their spectra will contain both the characteristic absorption peaks from the parent drugs and new and remarkable peaks resulted from P=O bonds and P-O-C bonds, which originally were hydroxyl groups, especially for the polysaccharide. Nuclear Magnetic Resonance (NMR) analysis is another widely adopted strategy due to the high resolution and brilliant accuracy of its spectra. When an electron-withdrawing phosphate group was introduced into the compounds, the chemical shifts of the carbon directly connected to them will be towards to the higher fields, which is crucial to determine the position where the phosphate group attaches.[28]

5.2. Chemical

The activation mechanism analysis is indispensable for drug innovation. With detailed and completed activation research, we will gain a full insight into how the prodrug functions stepwise in our physiological environment, directing our efforts to further refine its pharmacological efficacy. A study about HepDirect prodrugs, a novel strategy for targeting NTP production to the liver, included multidimensional observation via HPLC. While the rate of conversion would imply to what extent the prodrug will cleavage to release the active molecules, the CYP isoenzyme specificity was also evaluated to determine the medicine's discrimination of enzymes form the same family. More importantly, the products generated from incubating prodrugs will be analyzed to help identify the pharmacological target site and the process of the prodrug cleavage[29].

IC50 is the concentration of drug required for 50% inhibition, which is a quantitative parameter of the efficacy of a prodrug. A lower IC50 would be a signal of better drug candidates as it indicates higher bioactivity while suggesting a lower risk of side effects. For phosphate prodrugs, the enhanced capacity could be vividly displayed by IC50. For instance, Guo has reported that the IC50 of a phosphate derivatives of *Epimedium* Flavonoids is 30.8, nearly half of that of the parent compounds: 65.3. [22]

6. Conclusion

The prodrug strategy has now played an irreplaceable role in the exploration of original drugs, and among them, the ester derivatives with phosphorus atom are remarkable as their distinguishing properties. As synthesis methods of them are continuously developed, we obtain gradual understanding abouts their pros and cons. Nowadays, while some of them have been put into therapeutic practice, much more of them are still budding, with multiple techniques applied to analyze its potential as a drug candidate.

In seeking the top-class phosphorus-containing ester prodrugs, opportunities and risks coexist. As new natural products are increasingly discovered and exploited, phosphorylation on these molecules with pharmacological potency can drastically inspire our creation of the novel prodrugs, which will broaden the research of prodrugs by involving the modified novel natural product prodrugs at the early stages of drug discovery process. Moreover, to reduce the dosage of the drugs, the phosphorylated prodrugs should focus on enhanced bioactivities and stability in our body, necessitating research pursuing pharmacology. Finally, lowering toxicity and shielding the side effects should also never been spared, which is why it would be wise to delve into delicate choice and combination of potent and less toxic pro-moieties of the phosphate/phosphonate prodrugs. With continuous interest and attention in developing safer metabolites, coupled with increasing prodrugs in clinical trials and receiving FDA approval, the promising prospect of the P-containing prodrugs is certainly for sure.

References

[1] Diaz, M. E., Pena, R. E., Lobo, M., Schmid, R. A., Binkhathlan, F. H., & Tschinke, M. (2022). Advances in high-throughput mass spectrometry in drug discovery. *EMBO Molecular Medicine,* 15(e14850).

[2] Buckley, V., Thakkar, B., Patel, S. H., & Robins, M. R. A. (2020). High-Throughput Screening: today's biochemical and cell-based approaches. *Drug Discovery Today*, 25(10).

[3] Liu, R., Li, X., & Lam, K. S. (2017). Combinatorial chemistry in drug discovery. *Current Opinion in Chemical Biology,* 38, 117.

[4] Kennedy, J. P., Williams, L., Borchardt, T. M., Daniels, R. N., Wiest, D., & Lindsley, C. W. (2008). Application of combinatorial chemistry science on modern drug discovery. *Journal of Combinatorial Chemistry,* 10, 345.

[5] Venkatesh, S., & Lipper, R. A. (2004). Role of the development scientist in compound lead selection and optimization. *Journal of Pharmaceutical Sciences*, 89, 145.

[6] Rautio, J., Kumpulainen, H., & Heimbach, T. (2008). Prodrugs: design and clinical applications. *Nature Reviews Drug Discovery,* 7, 255.

[7] Rodriguez, J. B., & Gallo-Rodriguez, C. (2019). Chem. Med. Chem., 14, 190.

[8] Fang, S., Liu, Z., & Wang, T. (2023). Recent Advances of the Atherton-Todd Reaction. *Chinese Journal of Organic Chemistry,* 43, 1069.

[9] Kourounakis, S., & Pappas, G. (2022). Improvements, variations and biomedical applications of the Michaelis–Arbuzov reaction. *International Journal of Molecular Sciences,* 23(3395).

[10] Mao, X., Chen, J., & Yao, Y. (2024). Progress in phosphorylation of natural products. *Molecular Biology Reports, 51*, 697.

[11] Mehellou, Y., Singh, H. R., & Balzarini, J. (2018). The ProTide Prodrug Technology: From the Concept to the Clinic. *Journal of Medicinal Chemistry,* 61, 2211.

[12] Mehellou, Y., McGuigan, C., Brancale, A., & Balzarini, J. (2007). Design, synthesis, and anti-HIV activity of 2',3'-didehydro-2',3'-dideoxyuridine (d4U), 2',3'-dideoxyuridine (ddU) phosphoramidate 'ProTide' derivatives. *Bioorganic & Medicinal Chemistry Letters,* 17, 3666.

[13] Ying, H., Yao, J., Wu, F., Zhao, Y., & Ni, F. (2022). A mild and concise synthesis of aryloxy phosphoramidate prodrug of alcohols via transesterification reaction. *RSC Advances,* 12, 13111.

[14] Yao, J., Huang, C., Wu, F., Ni, F., & Zhao, Y. (2022). Synthesis of phosphoramidate prodrugs of phenolic natural products and drugs by ester exchange. *Synthesis*, 54, 3005.

[15] Chen, F., Huang, G., Yang, Z., & Hou, Y. (2019). Antioxidant activity of Momordica charantia polysaccharide and its derivatives. *International Journal of Biological Macromolecules,* 138, 673.

[16] Zhou, S.; Huang, G.Preparation, Structure and activity of polysaccharide phosphate esters. *Biomed. Pharmacother*. 2021, 144, 112332.

[17] Hu, H., Li, H., Han, M., Cao, Q., Liang, H., & Yuan, R. (2020). Chemical modification and antioxidant activity of the polysaccharide from *Acanthopanax leucorrhizus*. *Carbohydrate Research, 487*, 107890.

[18] Hu, S., Song, L., Pan, H., Hu, Y., & Gong, X. (2012). Thermal properties and combustion behaviors of flame retarded epoxy acrylate with a chitosan based flame retardant containing phosphorus and acrylate structure. *Journal of Analytical and Applied Pyrolysis,* 97, 109.

[19] Kim, W., Haws, H., Peterson, P., Whatcott, C. J., Weitman, S., Warner, S. L., et al. (2017). TP-1287, an oral prodrug of the cyclin-dependent kinase-9 inhibitor alvocidib. *Cancer Research,* 77(19), 5133.

[20] Mao, X., Chen, J., & Yao, Y. (2024). Progress in phosphorylation of natural products. *Molecular Biology Reports,* 51(2), 697.

[21] Patil, S., Lis, L. G., Schumacher, R. J., Norris, B. J., Morgan, M. L., Cuellar, R. A., et al. (2015). Phosphonooxymethyl prodrug of triptolide: synthesis, physicochemical characterization, and efficacy in human colon adenocarcinoma and ovarian cancer xenografts. *Journal of Medicinal Chemistry,* 58(24), 9334.

[22] Guo, C., & Huang, B. (2021). Study on preparation and activity of phosphates derivatives of Epimedium flavonoids. Guangdong University of Technology.

[23] Erion, M. D., van Poelje, P. D., Mackenna, D. A., Colby, T. J., Montag, A. C., & Fujitaki, J. M. (2005). Liver-targeted drug delivery using HepDirect prodrugs. *Journal of Pharmacology and Experimental Therapeutics,* 312(2), 554.

[24] Rebecca, E. F., Harrison, S., Ryan, J. M., Danielle, S., & Liu, G.-J. (2024). RSC Med. Chem., 15, 1973.

[25] Li, J., Wang, L., Han, M., Xiong, Y., & Liao, R. (2019). The role of phosphate-containing medications and low dietary phosphorus-protein ratio in reducing intestinal phosphorus load in patients with chronic kidney disease. *Nutrition & Diabetes*, 9(1), 14.
[26] Bharate, S. S., & Vishwakarma, R. A. (2015). Thermodynamic equilibrium solubility measurements in simulated fluids by 96-well plate method in early drug discovery. *Bioorganic & Medicinal Chemistry Letters*, 25(8), 1561.
[27] Xia, S., Zhai, Y., Wang, X., Fan, Q., Dong, X., & Chen, M. (2021). Phosphorylation of polysaccharides: a review on the synthesis and bioactivities. *International Journal of Biological Macromolecules*, 184, 946.
[28] Ruthes, A. C., Carbonero, E. R., Córdova, M. M., Baggio, C. H., & Santos, A. R. (2013). Lactarius rufus (1→3), (1→6)-β-D-glucans: structure, antinociceptive and anti-inflammatory effects. *Carbohydrate Polymers*, 94(1), 129.
[29] Erion, M. D., Reddy, K. R., Boyer, S. H., Matelich, M. C., & Jorge, G.-G. (2004). *Journal of the American Chemical Society*, 126(15), 5154.