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Progress in Molecular Imprinting—From Inhibition of Enzymatic Activity to Regulation of Cellular Pathways

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ABSTRACT

Molecular imprinting is a very powerful tool in life science. The research areas benefiting from a wide range of capabilities of molecularly imprinted polymeric nanoparticles (nanoMIPs) include sample preparation, extraction, sensing/detection, diagnostics, and drug delivery. Recently, a new member of this family—therapy/control of cellular reactions—has arrived. Within this newest field, so far, the design and synthesis of very selective enzymatic inhibitors/activators have been described. Since enzymes act as catalysts of biochemical reactions, nanoMIPs pose enormous potential in managing biological processes. Furthermore, in recent years, articles focused on influencing cellular pathways by either interaction with cell surface receptors or by inactivation of signal molecules have begun to appear. This strategy opens a new perspective for nanoMIPs application—as selective, inexpensive, and stable therapeutics. However, there are still a lot of questions to be answered and many issues that must be addressed before the practical implementation of nanoMIPs in the therapeutic area. Among the main challenges belong safety, biodegradability, biodistribution, and clearance of nanoMIPs from the organism as well as their reproducible large-scale production in accordance with quality control. This review aims to summarize the progress in nanoMIPs development enabling them to overcome main issues and increasing their competitiveness in the therapeutic area.

1 | Introduction

The major group of ever-expanding therapeutic agents makes up drugs based on small molecules. Small molecule-drugs are used to treat a variety of diseases and their mechanisms of action are quite diverse, for example, they can serve as effective enzyme inhibitors and allosteric modifiers or can target extracellular as well as intracellular receptors in the cytosol or nuclei. Nevertheless, the main issue linked with these types of drugs is the lack of specificity. Off-target interaction of small molecule-drugs with tissues, cells, and/or cellular components can lead to an undesirable influence on unintended cellular pathways. In some cases, off-target effects can be relatively harmless, however often; they cause serious adverse effects.

Approximately 35 years ago, advances in biotechnology enabled the synthesis of biological molecules in microorganisms and other living cells by using recombinant DNA technology [1, 2]. These innovations led to the expansion of a realm of medicaments by a wide range of biological formulations mainly based on proteins and peptides. Since then, enormous progress in biologics development and production has occurred. Today, several biological drugs belong to the top-selling drugs worldwide (e.g., Humira a monoclonal antibody that serves to treat rheumatoid arthritis) [3]. As mentioned above, proteins (especially antibodies) are the most common biological drugs [2]. They are large and complex biomolecules folded into unique three-dimensional structures selectively recognizing the antigen. In comparison with small molecule-drugs, biological therapeutics exhibit much higher specificity to the target

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structure which is connected with a lower probability of serious side effects. Nevertheless, even the use of protein-based therapeutics is not without a risk. The main problem regarding protein-based therapeutics relates to their immunogenicity (the tendency to trigger an unwanted immune response), which can even lead to life-threatening events [4, 5]. Another disadvantage is that the therapeutic proteins are usually thermosensitive, membrane impermeable, and subject to enzymatic degradation. Moreover, unlike many small molecule-drugs, due to low stability, protein drugs cannot be administered orally but require systemic routes of administration (e.g., injection) [6].

These issues lead to searching for novel approaches and materials that can serve as therapeutics. Molecular imprinting represents one of the most promising strategies for designing artificial influencers of cell regulation. NanoMIPs are synthetic receptors containing specific binding sites for a target—an imprinted molecule called a template. NanoMIPs are created by a process during which a polymer is formed around individual template molecules. Subsequently, the imprinted molecule is removed from the polymeric structure, which leads to the production of structures spatially and chemically complementary to the imprinted target. Thus, created nanoMIPs can specifically rebind their target into the created binding sites via a recognition mechanism that is very similar to antibody-antigen interactions.

In contrast to the small-molecule drugs, the nanoMIPs can be used for specific inhibition or activation of cellular pathways with high affinity and selectivity towards various targets (from small molecules to large structures as proteins). Also, in comparison to antibodies, nanoMIPs have a range of advantages—they are very stable, not subjected to enzymatic degradation, and their production is less financially demanding [7–10]. Moreover, the preparation process is very versatile enabling the tailoring of the physical–chemical properties optimal for the intended application.

Although the utilization of molecularly imprinted therapeutics is still in its infancy, it has the potential to kick-start the development of a new generation of drugs against a wide range of disorders and illnesses including cancer. In the last several years, great progress in nanoMIP-based enzymatic inhibitors or activators has been noted [9, 10]. Also, nanoMIPs serving as cell receptor blockers have been developed so far [10, 11]. These nanoMIPs were used, for example, to prevent cancer progression or the interaction between host cells and viruses.

Notwithstanding, the nanoMIP-based therapy seems to be a very attractive and rapidly developing scientific area. Currently, the therapeutic application of nanoMIPs has been discussed only in several review articles. Moreover, these papers were focused either on the application of nanoMIPs for targeted drug delivery [12–14] or cell imaging [15, 16]. The potential of nanoMIPs as therapeutics and agents for the regulation of cell metabolism and cell fate manipulation was, despite awe-inspiring results, mentioned only in passing. On the contrary, this review aims to provide an overview of the progress in the development of nanoMIP-based agents from simple enzymatic activity inhibitors to cell pathway regulators, and discusses their future potential.

To the best of the authors' knowledge, so far there is only one review paper not aiming at nanoMIPs as drug carriers but at the application of nanoMIPs as such as therapeutic agents; however, it is narrowly focused on cancer therapy [17]. This review aims to provide an overview of progress in nanoMIP-based drugs in various medical areas. Herein, nanoMIP-based therapy is divided into two groups: (A) nanoMIPs affect directly cellular pathways and cell behavior (as enzyme activity inhibitors/activators or receptor/ligand blockers), and (B) nanoMIPs ensuring the capture of xenobiotics and prevention of their interaction with a host organism leading to health issues (Figure 1).

2 | Challenges and Progress in Molecular Imprinting

Molecular imprinting is a rapidly advancing field driven by the demand for highly sensitive and rapid diagnostics, as well as safer and more efficient therapies. Although the advantages of nanoMIPs in diagnostic and therapeutic applications are evident, their competitiveness in in vivo strategies requires addressing several challenges. These include concerns regarding toxicity, blood circulation time, clearance, stability, reproducible synthesis, and production costs [7, 18, 19]. In this review section, we highlight both resolved issues and advancements in the development and preparation of nanoMIPs over the past several years. By exploring these topics, we gain insights into the progress made in tackling these challenges and shaping the future of nanoMIPs in clinical applications. An overview of the chronological sequence and the most significant milestones achieved in the development of nanoMIPs in the clinical field is depicted in Figure 2.

2.1 | NanoMIPs Production

To make the molecular imprinting economically viable and practical for commercial applications, the nanoMIPs synthesis must be efficient and cost-effective. Achieving this goal requires a careful consideration of several key factors. By thoughtfully selecting the template, optimizing functional monomers, and implementing automation and computer-aided design methods, cost-effective, highly selective, and scalable nanoMIPs can be developed to serve diverse industries, including diagnostics and therapy [20, 21]. As ongoing advancements in the nanoMIP synthesis techniques and design methodologies progress, the potential of nanoMIPs as a valuable tool in various scientific fields continues to grow exponentially.

2.1.1 | Selection of Functional Monomers

The selection of functional monomers is one of the most crucial factors affecting the selectivity of the resulting nanoMIPs. Currently, the most common approach for designing nanoMIPs that target a new compound relies on an experimental trial-and-error method.

However, considering the significant increase in the number of publications dedicated to the design of nanoMIPs using computational modeling, which rose by nearly 70% (from 229 in 2012 to 716 in 2022) over the past decade (according to the WOS database using topic phrases: "computational design" OR "molecular modeling" AND "imprint* polymer*"), it is evident that substantial

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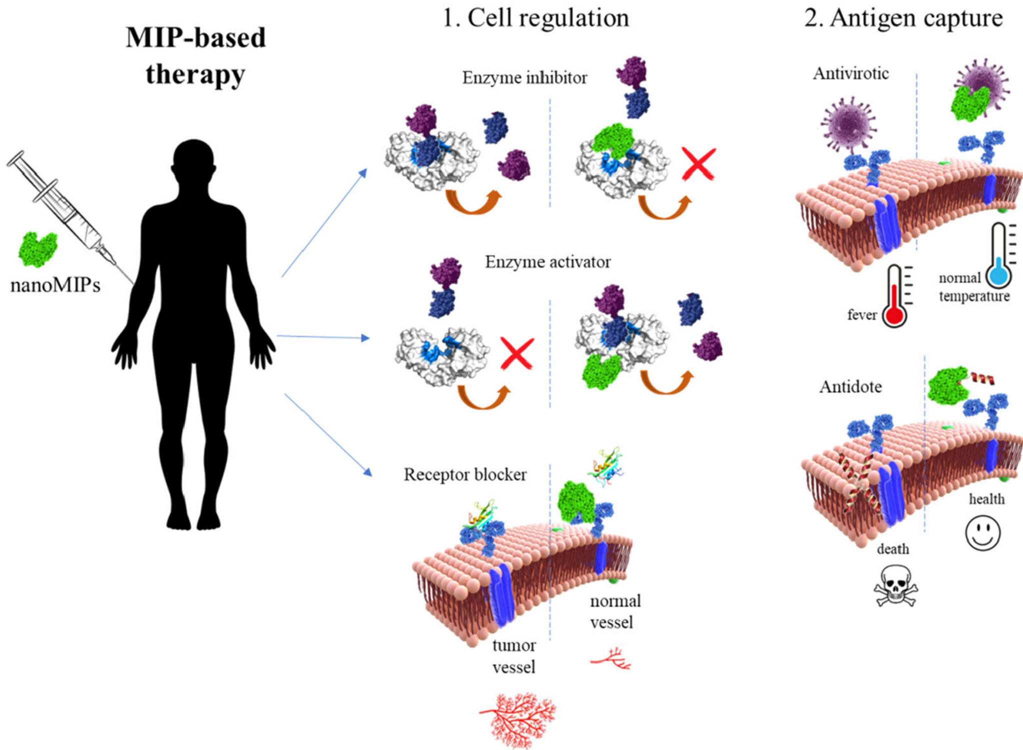

FIGURE 1 | NanoMIP-based therapeutics are divided into two groups: (1) nanoMIPs directly affecting cellular pathways and cell behavior, and (2) nanoMIPs capturing xenobiotics and preventing their interaction with the host organism leading to health problems. [Color figure can be viewed at wileyonlinelibrary.com]

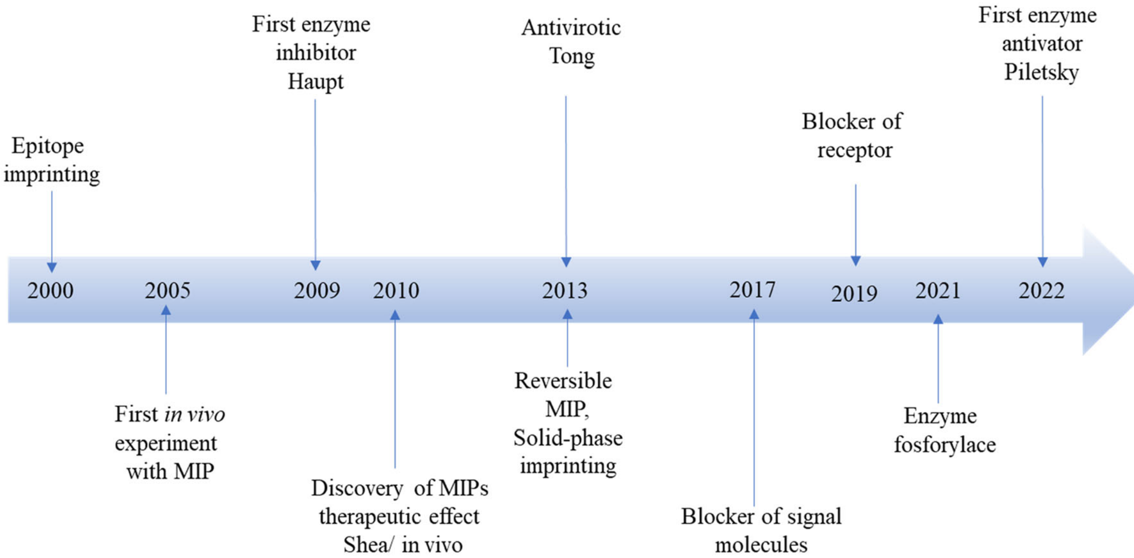

FIGURE 2 | Timeline representing the most important milestones achieved in the development of nanoMIPs in the clinical field. [Color figure can be viewed at wileyonlinelibrary.com]

and extensive efforts have been directed towards the computational design of the selection of the functional monomers.

2.1.2 | Selection of Template

Despite the significant advances in the technology of imprinted polymers, imprinting of larger structures such as proteins remains a considerable challenge. This challenge arises from several factors. Firstly, polyclonal nanoMIPs can be formed when dealing with structures such as proteins. This results in a wide range of binding sites with varying affinities and specificities. Additionally, maintaining the conformation and spatial orientation of native proteins during the polymerization presents serious difficulties. The large size of the imprinted structure also poses challenges in the removal of the template from the polymerized network, and the large binding sites may exhibit reduced selectivity, as they can interact with a range of

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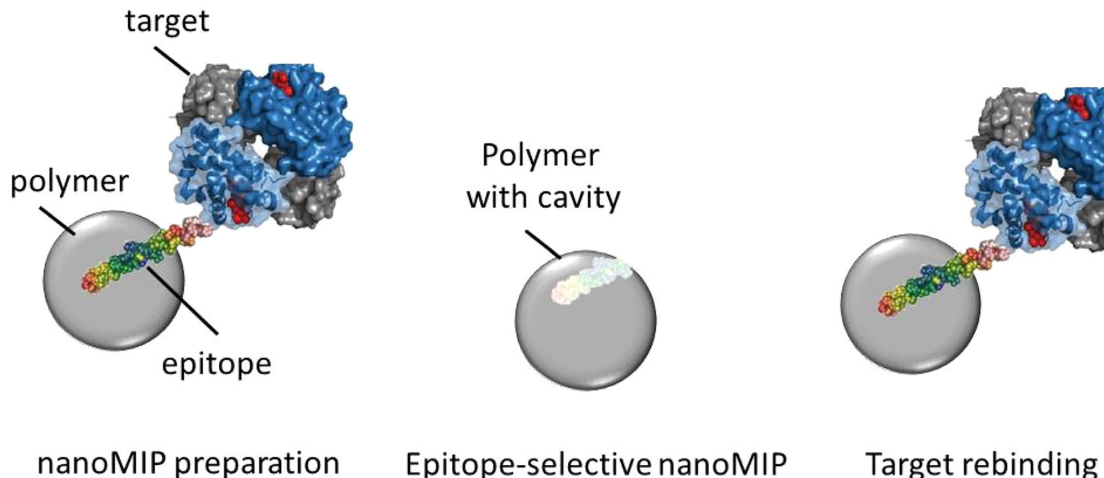

FIGURE 3 | Scheme of epitope imprinting approach principle. [Color figure can be viewed at wileyonlinelibrary.com]

smaller polypeptides. Moreover, the synthesis of nanoMIPs requires a significant amount of protein, and the cost of many proteins is relatively high [22, 23]. As a result, during the initial stages of development, inexpensive proteins that are readily available in large quantities, such as albumin [24], lysozyme [25], cytochrome c [26], or hemoglobin [27], were primarily used for proof-of-concept demonstrations.

In 2000, a groundbreaking strategy known as epitope imprinting was discovered [28]. Epitope imprinting (Figure 3) involves imprinting a small fragment that is characteristic for the chosen template, instead of imprinting its entire structure [29, 30].

There has been a growth in the number of publications dedicated to the synthesis of nanoMIPs by the epitope imprinting approach. Over the past decade, the number of such publications rose nearly 20 times, going from just 1 in 2012 to 19 in 2022 (according to the WOS database, using topic phrases: "epitope imprinting" OR "epitope imprint* polymer*"). However, it is essential to recognize that not all scientific articles explicitly mention working with epitopes in their title or keywords, which implies that the actual number of articles related to epitope imprinting may be higher than reported.

2.2 | Synthesis Approach and Automatization

Advances in computational methods and epitope imprinting have significantly simplified the design of nanoMIPs, making them more competitive with natural antibodies than ever before. Despite these remarkable developments, the commercial exploitation of nanoMIPs remains limited to a handful of products primarily used for analytical sample preparation through selective solid-phase extraction [31] and a few specialized sensors [32–34] applications. Broader adoption of this technology, especially in the fields of diagnostics and nanomedicine, has not yet been realized. NanoMIPs effectively serving as substitutes for antibodies must be prepared in a reproducible, controlled, and scalable manner.

There are several such approaches including precipitation, emulsion, bulk, or core-shell polymerization. However, this review will not delve into the pros and cons of each synthetic method, as detailed overviews addressing this matter have already been prepared [15, 35–38]. In 2013, a groundbreaking synthetic approach called solid-phase synthesis was developed to address this issue [39]. To enable the commercial application of nanoMIPs, precise control and optimization of the fabrication process are crucial to ensure excellent batch-to-batch reproducibility. In 2013, Poma et al. [40] developed an automated chemical reactor for solid-phase synthesis of nanoMIPs.

2.2.1 | Selectivity and Stability

Antibodies are renowned for their high binding affinities and strongly negative Gibbs free energies when recognizing their target species, enabling the detection of analytes at extremely low concentrations, even in complex matrices. This unique attribute has made them invaluable in medical diagnostics, drug development, and biosensing applications. While nanoMIPs also exhibit selectivity, they do not always match the performance of antibodies. Factors such as template density, affinity, and nanoparticle size can influence the selectivity of nanoMIPs. However, ongoing research and advancements in nanotechnology have led to significant improvements in nanoMIP properties, making them viable and competitive alternatives for certain applications. A study conducted by Smolinska–Kempisty et al. [41] compared the performance of nanoMIPs with antibodies in enzyme-linked competitive assays [41].

Antibodies, being sensitive biomolecules, require careful storage conditions to maintain their stability and activity. Unfortunately, their shelf life can be limited, posing challenges for certain applications. In contrast, nanoMIPs demonstrate superior stability and resilience. They can withstand harsh environmental conditions, such as extreme temperatures and pH levels, without significant loss of their binding capacity [42].

2.3 | In Vivo Application

2.3.1 | Toxicity

Currently, our knowledge of the nanoMIPs behavior in vivo remains inadequately understood. This is especially evident in

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the realm of understanding how the binding cavities of nanoMIPs influence processes such as internalization, biodistribution, and clearance within biological systems. This knowledge gap underscores the pressing necessity for further research aiming at unraveling the intricate phenomena that transpire once nanoMIPs are introduced into the bloodstream.

Kassem et al. [43] have presented a study describing the solid-phase synthesis of nanoMIPs, along with exploring their biodistribution, clearance, and cytotoxicity in a rat model. This investigation encompassed both intravenous and oral administration routes. The researchers specifically focused on the behavior of fluorescently labeled nanoMIPs that exhibit selectivity for trypsin in living organisms. The selection of trypsin as the target protein was deliberate because it lacks a direct association with the plasma membrane. Therefore, these nanoMIPs were not expected to accumulate on the surface of any particular cell type. Instead, their distribution pattern was expected to be based on variables such as size and surface properties.

The findings revealed that the administration of nanoMIPs at low doses, either through intravenous injection or oral ingestion, did not lead to swift entrapment by the reticuloendothelial system. These nanoMIPs demonstrated the ability to persist within tissues without inducing prominent toxic effects. The study revealed the nanoMIPs' ability to access all tested organs, including the liver, spleen, intestines, and brain. Clearance of these nanoMIPs from the body occurred through feces and urine within 168 h [43].

Additionally, the research encompassed a brief study of the nanoparticles' adjuvant properties. Notably, nanoMIPs tailored to target the cell surface protein epidermal growth factor receptor (EGFR) exhibited limited adjuvant properties when combined with the antigen ovalbumin. On the contrary, nanoMIPs designed for a control peptide exhibited no adjuvant characteristics. This outcome underlines the potential application of nanoMIPs in immunotherapy, particularly in cases where an increased immune response is desirable.

3 | Affecting of Cell Pathways by NanoMIP

In the 21st century, medicine is taking advantage of molecular science which enables targeting drugs directly to specific macromolecular biological targets whose bioactivity is pathogenic or associated with a disease. So far, numerous biological targets have been identified [44]. All current therapeutic targets can be subdivided into seven main classes (receptors, enzymes, hormones and factors, ion channels, nucleic acids, nucleic receptors and unknown) [45]. It is obvious that proteins, mainly receptors, are the predominant targets.

The most represented are G protein-coupled receptors that are commonly targeted by antihypertensive and anti-allergic drugs. Next, ligand-gated ion channels, commonly targeted by hypnotic drugs or sedatives, are the second receptor target class. Also, receptor of tyrosine kinases (e.g., epidermal growth factor receptor) targeted frequently by anticancer drugs are included [44].

Enzymes are the second largest group of target proteins (28%). The most common class of enzymatic drug targets is made up of hydrolases. Oxidoreductases and transferases follow. Other common enzyme targets include DNA polymerases, angiotensin-converting enzymes, and monoamine oxidases.

3.1 | Regulation of Enzymatic Activity by NanoMIPs

This part of the review aims at the therapeutical effect of nanoMIPs against different biological targets (specifically enzymes and receptors). Although the biggest group of biological targets is receptors, this overview will start with the use of nanoMIPs for enzymatic function regulation as enzymes were historically the first studied group of biological targets in molecular imprinting therapy. Moreover, this group clearly demonstrates the huge progress made in the last 15 years in the field of nanoMIP-based drugs.

Enzyme inhibition or activation belongs to one of the most important regulatory principles because it controls biological processes and metabolic reactions. Dysregulation of the enzymatic activity (e.g., by mutations in genes encoding enzymes) can lead to the development of pathological states. Enzyme inhibitors (EIs) affecting metabolic pathways through specific binding and blocking of the active or modulatory sites of enzymes, can serve to correct a metabolic imbalance or for blocking pathogenic enzymatic activity. Therefore, the development of EIs represents an important strategy in drug design [46, 47]. It is the reason why enzyme inhibitors play such a key role in clinical practice.

3.1.1 | Enzyme Inhibition

The synthesis of molecularly imprinted enzyme inhibitors (MIEIs) was the pioneering direction in the development of nanoMIPs therapeutics. An overview of nanoMIPs-based enzyme inhibitors/activators is shown in Table 1. MIEIs were described for the first time in ground-breaking work published by Cutivet et al. in 2009 [48]. In that publication, nanoMIPs were demonstrated to competitively inhibit trypsin with an inhibition constant (Ki) of 79 nM (three orders of magnitude lower than the chemical competitive EI- benzamidine). An original nanoMIPs synthesis strategy was used. The principle of the strategy is shown in Figure 4. This strategy employed a strong anchoring point—a low-molecular-mass inhibitor (4-aminobenzamidine methacrylate), which kept the contact between the functional monomers and the enzyme's active site during the polymerization process and simultaneously enabled incorporation into the growing polymer chain the presence of a polymerizable moiety. The principle of the approach is shown in Figure 4 [48].

The selectivity of trypsin inhibition provided by the developed MIEIs was verified using closely related enzymes, such as chymotrypsin and kallikrein. Although some inhibition of kallikrein by the nanoMIPs was observed, the effect was nearly insignificant in comparison with the inhibition of trypsin. This non-specificity might be because kallikrein has a similar substrate specificity as trypsin and, like trypsin, is inhibited by benzamidine derivatives [48].

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TABLE 1 | Overview of nanoMIP-based enzyme regulators.

Enzyme	Intra/extracellular enzyme	Reversibility	In vitro experiments	In vivo experiments	Imprinting strategy	Year	Ref.
Trypsin	E	N/A	No	No	Precipitation polymerization	2009	[48]
Trypsin	E	N/A	No	No	Surface imprinting	2013	[49]
Trypsin	E	Temperature	No	No	Surface imprinting	2015	[50]
DNase I	I	Ions	No	No	Surface imprinting	2013	[51]
APEA	I	Ions	No	No	Surface imprinting	2018	[52]
DNase I	I	N/A	Yes	No	Surface imprinting	2015	[53]
Catalase	I	N/A	Yes	No	Surface imprinting	2016	[54]
MMP-9	E	pH	Yes	Yes	Surface imprinting	2021	[55]
Trypsin/RNase	E/I	N/A	Yes	No	Solid phase synthesis	2021	[56]
Substrates of AMP-dependent protein kinase	I	N/A	No	No	Imprinting of cross-linked micelles	2021	[57]
Acetylcholinesterase	E	N/A	No	No	Solid phase synthesis	2022	[58]

This study has outlined the possibility of utilizing imprinting in a new and very attractive direction that has not so far been considered—the utilization of imprinted polymers in affecting cellular pathways. The therapeutical application of nanoMIPs can be the start of a novel branch of very selective, universal, and inexpensive medicinal drugs. However, it is necessary to overcome a considerable number of issues connected with nanoMIPs manufacturing and behavior within cells and organisms. Some of these problems, especially related to manufacturing, have already been solved. For example, the first described approach of MIEIs synthesis is associated with some issues such as time-consuming optimization of reaction conditions for achieving suitable binding properties and homogeneous particle sizes or low binding capacity caused by difficulties with the removal of the enzymes captured inside the deep cavities of nanoMIPs, and so forth. And it was also the reason why the interest in MIEIs stopped for some time. These problems were solved by introducing the core-shell imprinting approach (Figure 5) [49]. This approach is based on a synthesis of a thin layer of the molecularly imprinted polymer containing target-specific cavities on the surface of a solid carrier such as metal or silica particles. The advantage of this method is the uniform nanoMIP size and prevention of the template being stuck or buried deep inside the polymer leading to easier template removal.

One of the first works, that utilized the approach of core-shell imprinting for MIEIs synthesis, is the work published by Zhang et al. [49]. In that publication, MIEIs selective for trypsin prepared by a controlled surface molecular imprinting approach prepared on the surface of small microspheres are introduced [49]. As in the case of the MIEIs developed by Cutivet et al., aminobenzamidine was used to ensure the oriented imprinting of the enzyme and to increase the inhibition efficiency. The inhibition efficiency of trypsin by the imprinted microspheres was four times higher in comparison with MIEIs prepared by Cutivet et al. [48, 49]. These results confirm that surface imprinting, in comparison with classical bulk polymerisation, leads to increased nanoMIPs efficiency. This was the milestone that led to the progression of MIEIs synthesis and their advanced application.

The above-mentioned works described irreversible MIEIs containing a small chemical enzyme inhibitor serving as a polymerizable anchor. Later, the reversible MIEIs whose action is triggered either by a certain physiological status or by external stimuli started to appear. The first study on this topic presented a programmable inhibition of a model enzyme (i.e., trypsin) activity by using a thermal-responsive smart molecularly imprinted nanocomposite composed mainly of N-isopropyl acrylamide [50]. Such responsive materials could specifically recognize and capture trypsin maintaining most of its enzymatic activity (70%) at normal body temperature and at abnormally high body temperature triggering the inhibition of its enzymatic activity (10% of its original value) due to the hydrophilic–hydrophobic conversion of the imprinted cavities [50].

In another study, the molecularly imprinted layer was synthesized on a surface of magnetic particles for specific and reversible blocking of model enzyme deoxyribonuclease I activities [51]. The resultant MIEIs enabled complete blocking of the activity of the target enzyme via selective adsorption in cell lysates and also the quantitative release of the bound enzyme under mild conditions with the assistance of metal ion

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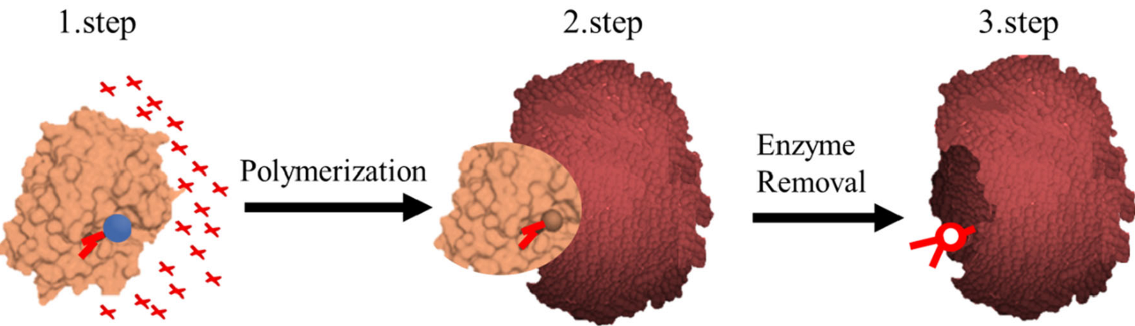

FIGURE 4 | Scheme of nanoMIP enzymatic inhibitor synthesis. In the first step, the enzyme comes into contact with the anchoring monomer and comonomers, where polymerization subsequently takes place. In the second step, the binding site of the substrate is formed. Subsequently, the enzyme (template) is removed. In the third step, a specific binding site with inhibitory properties is revealed. Adopted from [48]. [Color figure can be viewed at wileyonlinelibrary.com]

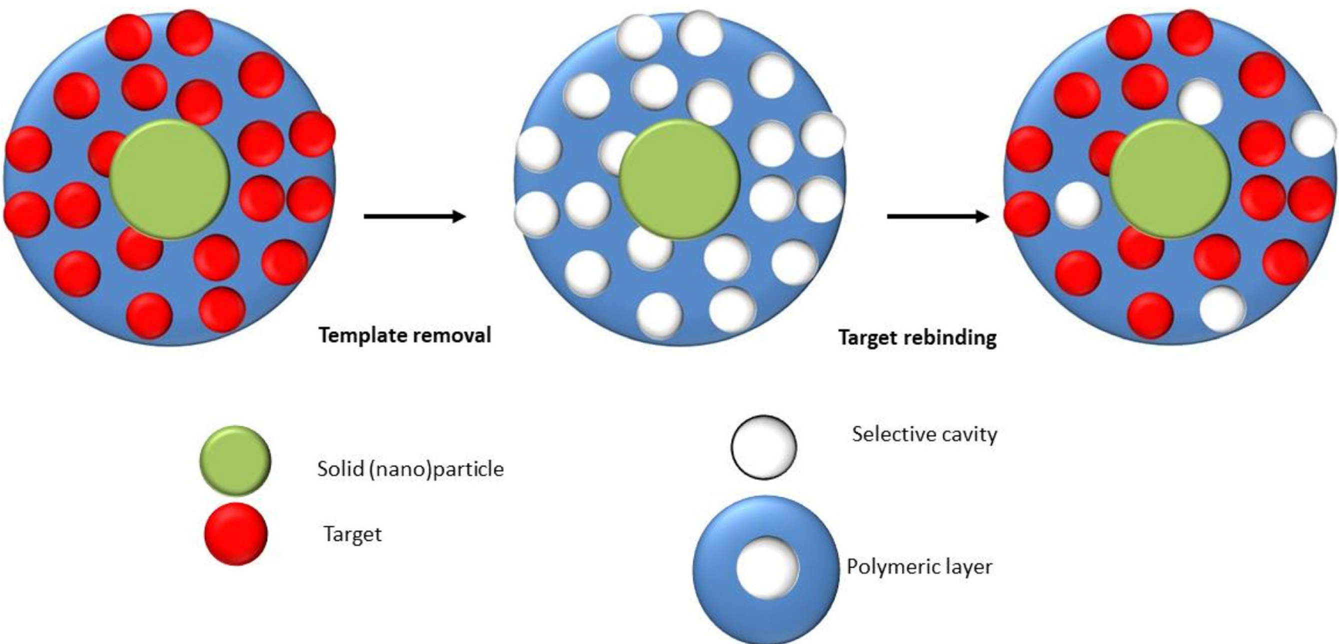

FIGURE 5 | Scheme of core-shell imprinting approach principle. [Color figure can be viewed at wileyonlinelibrary.com]

cofactors (in the presence of Ca^{2+} and Mg^{2+}, 90% enzyme recovery occurs) [51].

The same reversible approach to the regulation of the enzymatic activity by using of MIEIs was also used in work published in 2018 by Zhai et al. [52]. The authors focused on the development of MIEIs for human apurinic/apyrimidinic endonuclease/redox effector factor 1 (APE1) which is the DNA repair enzyme that helps to keep the stability of the genome. In normal tissues, APE1 is mostly localized in the nucleus, while in aggressive tumors; the predominant localization of APE1 is in the cytoplasm [52]. Therefore, the inhibition of cytoplasmic APE1 can serve as an effective tool in cancer therapy. The study introduced a polydopamine bionanocomposite on the surface of silica-coated magnetic nanoparticles, prepared via surface molecular imprinting, and enabling the reversal of the inhibition of the enzyme APE1, by using avidin as a bioaffinity ligand. MIEIs synthesized in that publication suppressed the enzymatic activity by more than 70%. Moreover, a mixture of Mg^{2+} and Mn^{2+} enabled the recovery of the bound protein as high as 80%. These artificial reversible nanoinhibitors have great potential for targeted cancer therapy because they can both inhibit and reactivate the activity of enzymes [52].

When the preparation and reversibility of MIEIs were examined in detail, the studies of MIEIs on the cell lines followed. The first in vitro experiment with MIEIs was performed in 2015 by Liu et al. [53]. In their work, authors demonstrated fluorescent PEG-modified surface imprinted polymers prepared on magnetic nanoparticles. These MIEIs were readily taken up by living cells (after 30 min were clearly seen in the cytoplasm) and enabled selective and effective blocking of the enzyme activity of DNase-I in vitro (approximately 35% inhibition) without nonspecific interactions with other biomolecules and low cytotoxicity. This study had a huge impact because confirmed the presumption of usability of MIEIs in living cells [53].

In another interesting study, Chen et al. [54] introduced a new idea for the synthesis of a multifunctional nano-platform composed of a magnetic core enwrapped by catalase (CAT)-

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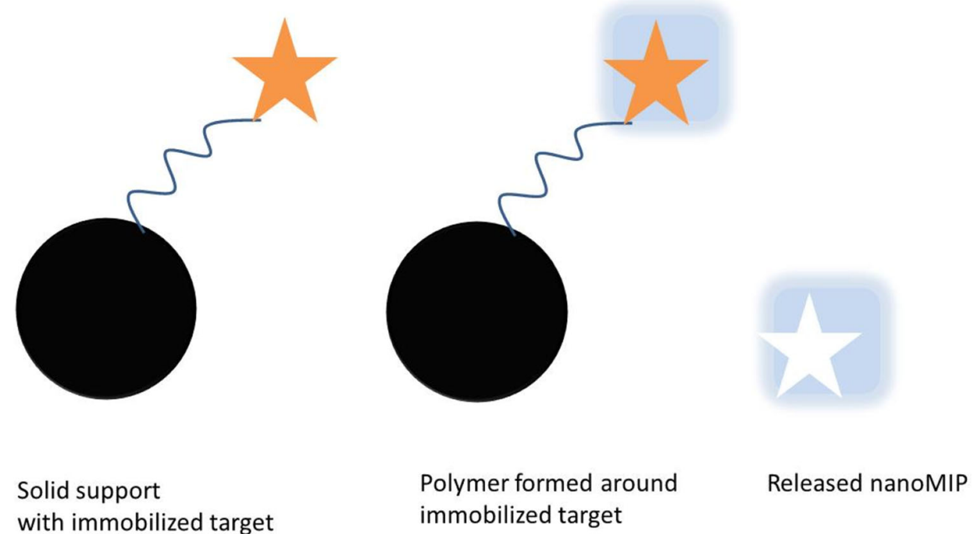

FIGURE 6 | Scheme of solid-phase synthesis principle. [Color figure can be viewed at wileyonlinelibrary.com]

imprinted fibrous shell for cancer therapy. The principle of that work is based on the assumption that a high level of intracellular reactive oxygen species (ROS) causes oxidative damage to cell components (such as lipids, proteins, and DNA) leading to the triggering of cell apoptosis. However, tumor cells can regulate the intracellular ROS level through the rapid enzymatic transformation of overproduced toxic H_2O_2 to nontoxic water and oxygen by CAT. The study demonstrated that developed nanoMIPs can selectively inhibit the bioactivity of CAT in the tumor cells, which caused a dramatic elevation of the H_2O_2 level (by more than 65% after 100 s exposure). Moreover, simultaneously the core of nanoMIPs was releasing Fe^{3+} ions that catalyze the conversion of H_2O_2 to a stronger oxidizing agent—hydroxylic radicals. Eventually, the concentration of ROS in tumor cells increased to a lethal level which led to the apoptosis. It was found that after 24 h of nanoMIPs exposition the accumulated Fe^{3+} reached 70% at pH 5.4% and 55% at pH 6.5, but only 10% at pH 7.4. Obtained results proved that because of an acidic intracellular environment within the tumor cells, nanoMIPs effectively affected these cells while nontumor cells were not influenced by the nanoMIPs [54].

Until 2016, the core-shell imprinting method was used, which can suffer from non-oriented imprinting and an unknown number of binding sites, which reduces reproducibility. In 2016, solid-phase imprinting (Figure 6) [4] was introduced, based on covalent immobilization of the template onto a solid support (e.g., glass beads). This approach ensures uniform template orientation and homogeneous binding sites, which improves reproducibility [4].

Unlike traditional methods that require complex purification steps, solid-phase imprinting works like an affinity separation column. Unreacted monomers and polymers with low affinity are removed at room temperature, while high-affinity nanoMIPs remain bound. They are subsequently selectively released by increasing the temperature. This process can be easily automated, and the resulting nanoMIPs have uniform binding properties [59].

In 2021, the first MIEIs prepared by this method were developed by Cutivet et al. [48]. It was found that this approach ensures that the active site of the enzyme remains accessible during the polymerization process leading to the synthesis of MIEIs with high specificity and potent inhibitory effects. In a study [56], two types of MIEIs, for inhibition of extracellular enzyme—trypsin and intracellular enzyme—angiogenin, were synthesized. The developed MIEI-trypsin had a very low inhibition constant (Ki) of 3.4 nM. Moreover, an in vitro assay was performed to investigate whether MIEI-trypsin can protect a cell against tryptic digestion-induced extracellular matrix (ECM) lysis. It was found that L-02 cells in the presence of trypsin began to detach within a period of 10 min. In the presence of MIEI-trypsin, no significant ECM lysis or cell detachment was observed within 60 min. It suggests that MIEI-trypsin can be applied in the treatment of active trypsin-dependent cell injury. The angiogenin MIEI (MIEI_RNase) inhibited cancer cell proliferation by suppressing the ribonuclease activity of angiogenin and decreasing the level of intra/extracellular angiogenin. It was found that the inhibition effect of MIEI_RNase was higher in comparison with the natural ribonuclease inhibitor. Moreover, the inhibitory property of MIEI_RNase was functional for 5 days, which indicates these MIEIs can serve as a stable therapeutic drug for long-acting cancer therapy. The work demonstrated the versatility of MIEIs for both enzyme inhibition and cell fate manipulation, showing their great potential as therapeutic drugs in biomedicine [56].

In 2021, the first study of MIEIs behavior in vivo was carried out [55]. In that work, a thin imprinted layer was prepared by reversible addition-fragmentation chain transfer polymerization on a surface of gold nanorods using Matrix metalloproteinase-9 (MMP-9) as the template. MMP-9 is an enzyme that participates in cancer cell migration and the regulation of the tumor microenvironment. No obvious change in the body weight was observed in any mice group with MIEIs in vivo treatment, and histology analysis of the mice organs after the 21 days of the treatment proved that there was no obvious organ damage and cellular structure changes in all major organs. This confirmed

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that prepared MIEIs did not cause any severe biological toxicity. Moreover, the prepared MIEIs suppressed the migration and growth of metastatic tumors (54% inhibition of tumor growth after 19 days). A better tumor inhibition rate was achieved in nude mice with a 4T1 tumor model compared to the MMP-9 monoclonal antibody (44% inhibition of tumor growth after 19 days) and antiangiogenic drug TNP-470 (40% inhibition of tumor growth after 19 days). The work provided the proof that nanoMIPs can be effectively used for cancer therapy in live organisms [55].

In some cases, it is impossible to inhibit the enzyme itself, owing to its multifunctional role in living systems. For example, kinases provide the regulation of a wide number of cell mechanisms by protein phosphorylation and their uncontrollable activity can lead to the development of tumor diseases. Therefore, kinases are often targets for anticancer drugs. However, the traditional inhibition of this enzyme can lead to unintended consequences owing to its multiple roles. Therefore, the inhibition of the enzyme's substrate (instead of the enzyme itself) is employed. However, the inhibition of phosphorylation of highly similar peptide sequences is very difficult to achieve.

A completely new approach to enzyme-substrate inhibition was described in the work of Li et al. [57]. Imprinted nanoparticles prepared in that study enabled the utilization of the nanoMIPs, which are able to bind peptides with high affinity and specificity. The peptides contained the most abundant consensus motif (RRXS (X = a variable amino acid): Kemptide (7, LRRASLG),18 β2-adrenergic receptor peptide (8, TGHGLRRSSKFCLK),19 pyruvate kinase peptide (9, PAGYLRRASVAQLT), 20 and cardiac myosin-binding protein-C peptide (10, FRRTSLAGGGRRISDSHE)). Thus, the phosphorylation of chosen proteins was controlled. The binding of protein to nanoMIPs can compete with protein–enzyme interactions within a multi-domain kinase. In this way, a controlled posttranslational modification (PTM) can be performed in a previously unavailable manner. Cyclic AMP-dependent protein kinase (PKA) was used as a model kinase. The PKA is capable of identification and phosphorylation of over 100 physiological substrates. Four substrates containing the most abundant consensus motif RRXS (X = a variable amino acid) were used for the phosphorylation inhibition study in the work.

Specifically, the substrates included: Kemptide (No. 7, LRRASLG), β2-adrenergic receptor peptide (No. 8, TGHGLRRSSKFCLK), pyruvate kinase peptide (No. 9, PAGYLRRASVAQLT), and cardiac myosin-binding protein-C peptide (No. 10, FRRTSLAGGGRRISDSHE). Peptides No. 7 and 9 have identical consensus motifs, and peptide No. 10 has two phosphorylation sites. The binding affinities of the nanoMIPs to their corresponding peptides were measured by isothermal titration calorimetry and high-performance liquid chromatography. For peptide No. 10, two types of MIPs were prepared, each selective for one of the two phosphorylated areas (a, b). It was found that the K_D values for nanoMIPs (No. 7–No. 10) were one to several orders of magnitude smaller than the K_M values for a typical kinase (19–550 nM). A mixture of all four peptides was tested to control the inhibition of PKA phosphorylation. It was found that when nanoMIPs for proteins No. 8 and No. 9 were simultaneously added to the mixture of all peptides and PKA, only about 6% of peptide No. 8% and 5% of peptide No. 9 were phosphorylated while peptides No. 7 and No. 10 were phosphorylated completely. Subsequently, the bisphosphorylation of one peptide (No. 10) was studied. When only nanoMIPs for peptide No.10a or No. 10b were present, the bisphosphorylated product of peptide No. 10 was not formed while the formation of desired monophosphorylated products was observed. When nanoMIPs for both peptides (No. 10a, b) were added to the reaction mixture, neither mono- nor bisphosphorylated products were formed. This proves the precise control of peptide phosphorylation using synthetic materials. The most beneficial feature of this approach is its versatility—the possibility to protect single or multiple areas of the chosen protein against intermolecular or intramolecular phosphorylation. Moreover, the possibility to inhibit a particular phosphosite while other sites in the same substrate can undergo phosphorylation (Figure 7) seems to be very beneficial. Given the importance of phosphorylation of proteins in biology, it is expected that these materials enable researchers to control PTM in a previously impossible manner and thus move cancer therapy a step forward [57].

3.1.2 | Enzyme Activation

While nanoMIPs for inhibition of the enzymatic activity are becoming relatively common, nanoMIPs-induced increase of the enzymatic activity (MIEAs) is very rare. In 2022, Piletsky et al. [58] introduced MIEAs for the first time and so far it is the only publication focusing on this topic. In that work, an innovative epitope mapping technique was used to identify the ideal surface epitopes of acetylcholinesterase (AChE), which enable to affect the shape or dynamics of that enzyme's active site. The solid-phase imprinting approach was subsequently used to synthesize MIEAs selective for these epitopes. All MIEAs exhibited an excellent affinity to AChE, with calculated K_D values in the nanomolar range between 0.4 nM (for FRF-nanoMIP) and 78.6 nM (for FGE-nanoMIP). The enzyme activation abilities of nanoMIPs were investigated in the presence of malathion (an organophosphate pesticide causing irreversible inhibition of AChE). It was found that nanoMIPs (for LAL and FGE epitopes) had a beneficial effect on retaining and restoring the enzymatic activity. The influence of malathion, reducing the activity of AChE to 11% of its original activity, was in the presence of developed nanoMIPs almost entirely negated. Presented MIEAs are envisaged as novel therapeutics for both Alzheimer's disease and organophosphate intoxication through allosteric inhibition and activation. This method of enzymatic activation shows promise in treating various enzyme deficiency diseases and solving problems with xenobiotics that affect enzyme function [58].

3.2 | NanoMIPs as Receptor Modulators

Receptors are integral membrane proteins that upon interaction with extracellular ligands trigger intracellular signaling cascades. Since receptors control a wide range of biological events, they belong among the most common drug biological targets. Traditionally, drug development focuses on either promoting or preventing receptor activation. In a case when the biological response of the receptor is activated the drug is called an agonist; in the reverse case (when the activity of the receptor is blocked or dampened), the drug is called an antagonist. The

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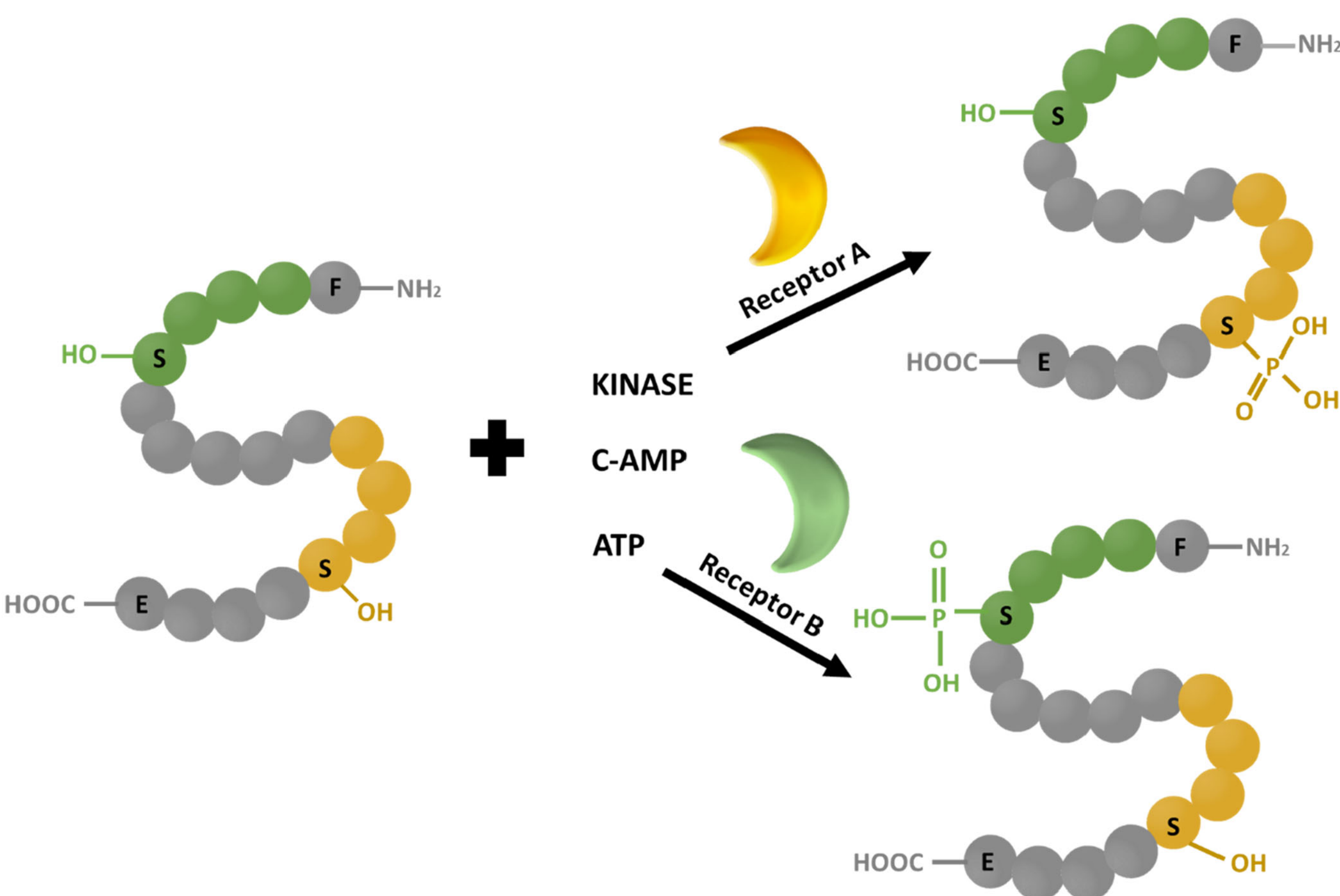

FIGURE 7 | Control of kinase activities with the ability to inhibit a specific phosphosite. While other sites in the same substrate may undergo phosphorylation. Adopted from [57]. [Color figure can be viewed at wileyonlinelibrary.com]

activity of the receptors can be affected by directly targeting the receptor protein or blocking its extracellular ligand.

In the case of molecular imprinting, the therapeutics affecting the activities of a receptor are still in their infancy. The first effort to influence the receptor's activity, presented in 2017, was focused on the signal ligand blocking the receptor [60]. Only in the last few years, publications aiming at directly inhibiting the receptor have appeared [61]. All the nanoMIPs-based therapeutics developed so far were focused on the treatment of cancer. Despite how novel this area is, the obtained results are very optimistic. The following part of the review is dedicated to a more detailed description of the individual discoveries in molecularly imprinted therapeutics affecting receptor's activity. Firstly, nanoMIPs-based inhibition of signal molecules is described, following the approaches for direct receptor inhibition.

3.2.1 | NanoMIPs as a Blocker of Signal Molecules

Angiogenesis is a physiological process, during which new blood vessels are formed from pre-existing vessels. In a healthy individual, this process occurs in embryonic development or in adulthood during wound healing. However, angiogenesis also plays a significant role also in the tumor development in cancer. In this case, it participates in the formation of new blood vessels that supply the emerging tumor and nourish it.

Among the effectors involved in angiogenesis belong the vascular endothelial growth factor (VEGF), which has been intensively investigated over the years for its crucial role in this process [60]. VEGF stimulates the proliferation of tumor cells together with the proliferation and differentiation of endothelial cells and is overexpressed in many invasive types of cancers (e.g., breast, colorectal, gastrointestinal, etc.) [60, 62]. Inhibition of the VEGF signaling blocks the angiogenesis in the tumors and changes or destroys tumor vessels. This makes VEGF a very interesting drug target.

In 2017, two works were published in parallel both focusing on the inhibition of VEGF by nanoMIPs [60, 61]. In the first study published by Cecchini et al. [60], nanoMIPs selective for a surface epitope of VEGF were developed. These nanoMIPs were produced by the solid-phase synthesis and coupled to quantum dots (QDs). Developed QD-nanoMIPs showed an excellent affinity for the VEGF epitope, with K_D in the nanomolar range (1.56 nM). The ability of nanoMIPs to target the tumor tissue was confirmed in vivo in zebrafish embryos with xenotransplanted human malignant melanoma cells. Two tumor models were prepared by injecting two human melanoma cell lines in a yolk of zebrafish embryos (i.e., WM-266 overexpressing VEGF(+)) and A-375 with low expression of VEGF(−)). Data analysis highlighted a statistically significant difference in QD-nanoMIPs injected in VEGF(+) and VEGF (−) embryos. This confirms that QD-nanoMIPs were able to target the tumor cells in vivo. Moreover, no toxic effects of QD-

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nanoMIPs resulting in reduced viability and/or alterations of normal development of zebrafish embryos were observed [60].

While the first study was rather focused on studying the specific recognition of VEGF and tumor targeting by nanoMIPs in vivo [60], the second study, published by Koide et al. [61], aimed mainly at the biological effect of nanoMIP-blocked VEGF. In that study, it was found that created nanoMIPs selective for VEGF were able to inhibit the binding of the signaling protein to its receptor (i.e., VEGFR-2). This prevented the receptor phosphorylation, and thus inhibition the VEGF-dependent growth, tube formation, migration, and invasion of cells (HUVEC cell line) in vitro. The synthesized nanoMIPs were nontoxic and did not exhibit off-target activity. Moreover, nanoMIPs inhibited VEGF-dependent angiogenesis in a Matrigel plug in living mice. These results were followed up by an in vivo study published 2 years later [63] focused on the behavior and the fate of nanoMIPs selective for VEGF in organisms. It was found that the nanoMIPs rapidly (60 min after administration) accumulated in the liver (50%) and in the kidney (20%). Accumulation in the tumor was approx. 0.4% at 60 min after the injection. The clearance of more than 97% of particles from the body took 4 weeks. Prepared nanoMIPs had only a little affinity to plasma proteins and did not exhibit reduced affinity to the target biomacromolecule in the bloodstream. It was proven that the nanoMIPs inhibit angiogenesis in vivo without triggering inflammation and/or bleeding. Further, the synergic effect of doxorubicin (DOX) and developed nanoMIPs was studied. It was found that the combined therapy by nanoMIPs and DOX significantly enhanced the antitumor effect. This study provides important information about nanoMIPs' safety, biodistribution, and clearance in vivo indicating that nanoMIPs provide a huge potential as cost-effective, stable, functional materials for therapeutic applications.

Another receptor signal molecule that was blocked by nanoMIP-based therapeutics was testosterone (TSTO). TSTO is a naturally occurring androgenic steroid that maintains muscle strength and bone density [64, 65]. However, in the 1940s it was found that TSTO can cause some pathologic conditions including the growth of prostate cancer [64]. Therefore, drugs that block the action of TSTO can be used in the treatment of advanced prostate cancer. Tang et al. developed nanoMIPs for TSTO (LOD 0.05 $ng\ mL^{-1}$) which at a concentration of 160 $\mu g\ mL^{-1}$ successfully blocked the TSTO-androgen receptor (AR) pathway within 24 h [64]. The selectivity of the nanoMIPs was verified using four structural TSTO analogs (dihydrotestosterone, progesterone, methyltestosterone, and testosterone propionate). In brief, it was proved that the nanoMIPs could enter the prostate cancer cells LNCaP and C4-2, specifically recognize and bind intracellular TSTO, and thus inhibit the TSTO-AR cascade-related functions as well as the growth of androgen-dependent prostate cancer cells through suppression of the cell cycle progression [64].

In 2021, Zhou et al. [66] published a study focusing on the inhibition of PD-1 transmembrane protein receptors by nanoMIPs. PD-1 receptor is predominantly expressed on the surface of immune cells including T-cells, B-cells, natural killer (NK), and so forth. PD-1 ligand (PD-L1) has the task of regulating the T-cell-mediated immune response in peripheral tissues, thus reducing the damage to the tissues possibly caused by a strong immune response [66]. However, it was demonstrated that the PD-1 signaling pathway can also be exploited by tumor cells to evade the antitumor immune response; therefore, blocking the PD-L1 can advance the cancer treatment [66, 67]. Within the study, the PD-L1 blocking strategy utilizing nanoMIPs was developed to activate the antitumor immunity of T-cells. Despite the protein nature of PD, in this case, the target part of the ligand was not a peptide but glycans, which were enzymatically digested, purified, and used as templates for the imprinting. Boronic acid, well-known for its ability to willingly and selectively interact with diols (e.g., saccharides), was chosen for therefore boronate-affinity-controllable oriented surface imprinting nanoMIPs synthesis. The surface of these nanoMIPs was functionalized with a sialidase, enabling the effective cleavage of tumor surface sialoglycans which enabled the T-cell infiltration enhancement. In vitro tests proved that nanoMIPs with the dual function (i.e., PD-L1 blockage and desialylation) in combination with T-cells significantly inhibited the proliferation of PD-L1-positive MDA-MB-231 cells. The in vivo therapeutic effect of nanoMIPs was examined in BALB/c nude mice with implanted MDA-MB-231 tumors. A tumor growth study showed that nanoMIP treatment inhibited tumor growth as the weight of the tumor after 10 days was lower by more than 20% in comparison with the control (i.e., PBS). The study provided a useful method to improve the treatment effect of immune checkpoint therapy [66].

3.2.2 | NanoMIPs as Direct Receptor Antagonist

Cadherins are a family of cell adhesion receptors. They play a significant role in tissue homeostasis, including responsibility for cell–cell adhesion, tissue morphogenesis, and differentiation [68]. However, any dysfunction or destabilization of cadherins may lead to tumor progression [68]. Therefore, one of the promising therapeutic strategies to fight cancer growth and prevent metastasis is based on the development of drugs that inhibit cell–cell adhesion mediated by cadherins.

Rangel et al. [69] designed nanoMIPs capable of recognizing the N-terminal peptide sequence (DWVIPPI), which is the site responsible for adhesion in E- and N-cadherins [69]. The created nanoMIPs were able to recognize this cadherin peptide epitope at the surface of HaCaT, MCF-7, and HeLa cells. Moreover, cell aggregation assays showed that the nanoMIPs inhibited, and even completely abrogated the cell–cell adhesion of HeLa cells. The nanoMIPs also enabled the disruption of preformed tumor spheroids and even inhibited the cancer cell invasiveness of HeLa cells in vitro. This means that nanoMIPs could loosen a dense extracellular matrix in the tumor microenvironment, which made drug penetration through the tightly packed tumor cells easier and thus their therapeutic effect is increased. These biocompatible nanoMIP-based anti-adhesive agents may potentially be used as immunotherapeutic or sensitizing agents to enhance the antitumor effects of chemotherapy [69].

Although both E- and N-cadherins are involved in cancer development depending on the cell and tissue type [69], targeting and inhibiting only one of these two cadherins is also interesting as shown in a follow-up study published by Rangel

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et al. [69]. In that work, the authors used the epitope sequence (CAHAVDINGC) as a nanoMIPs template. This sequence is responsible for the subtype specificity of N-cadherin and is involved in the cadherin-mediated interaction. Results indicate that the selected epitope CAHAVDINGC generated nanoMIPs selective for N-cadherin. It was also found that the nanoMIPs selective for the epitope sequence CAHAVDINGC (IC_{50}: 25 nM) were as efficient as the nanoMIPs selective for DWVIPPI21 (IC_{50}: 20 nM) in blocking cancer invasion in vitro. The presented results demonstrated that nanoMIPs were able to influence complex biological processes such as modulating cadherin activity which advances the treatment of diseases associated with dysfunctional cadherin mechanisms [70].

The second investigated receptor in nanoMIP-based therapy was the HER2 receptor which plays a critical role in cell proliferation, differentiation, and survival. However, in some cases, its overexpression can occur which is often related to various diseases such as breast cancer, gastric cancer, etc [71, 72]. This pathological state is associated with a poor survival prognosis and with the expansion and dissemination of cancer [72]. HER2 signaling pathway, which regulates cell proliferation, survival, migration, angiogenesis, and metastasis, starts by heterodimerization of HER2 with HER1 or HER3. This is preventing dimerization of HER2 with other HER1/HER3 proteins providing an effective treatment for HER2-positive breast cancer.

In 2019, nanoMIPs blocking the HER2 signaling pathway and thereby inhibiting the growth of HER2(+) breast cancer were introduced [72]. In this case, similarly, as in the case of PD-L1 inhibition, the glycan-imprinted principle was used. Boronate-affinity-controllable oriented surface imprinting was chosen for the nanoMIPs synthesis. Prepared nanoMIPs were able to selectively recognize HER2 receptors. Binding to HER2, nanoMIPs created a steric hindrance preventing the dimerization with other members of the HER family. This led to blocking the HER2 signaling pathways and thereby inhibiting the growth of HER2(+) breast cancer. In vitro experiments demonstrated that the nanoMIPs can specifically target HER2 and inhibit cell proliferation by 30%. In vivo experiments showed that the mean tumor volume of the nanoMIP-treated group was half in comparison with the non-treated group. That study demonstrated a novel possible strategy to treat HER2(+) breast cancer which presents the promising potential of nanoMIPs in cancer therapy [72].

4 | NanoMIPs as Antigen Inactivator

Approximately 75% of drugs are targeted to defined protein structures of human origin. The rest comprises other targets including molecules by pathogens, such as viral and bacterial proteins and extracellular macromolecules or DNA [73].

The following part of the review aims at the therapeutical effect of nanoMIPs against pathogenic targets (e.g., viruses and venoms).

4.1 | NanoMIPs as Antivirotics

Glycoproteins are major components of the outer surface of viruses. These glycoproteins mediate most of the interactions of viral pathogens with their hosts and usually elicit the immune response in the human body [74]. Just like the host's immune system can specifically recognize invading viruses via these glycoproteins, nanoMIPs can be designed to use similar principles to recognize viruses and/or their virulence factors [75]. In this part of the review, the nanoMIPs that selectively recognize whole viruses and/or viral surface components (proteins/peptides) are discussed. These nanoMIPs can be effectively applied not only in diagnostics but also directly in the treatment of viral infections. The overview of the imprinted polymers serving as antivirotics is shown in Table 2.

The first study on the application of nanoMIPs antiviral agents for infectious disease treatment was published in 2013 by Sankarakumar et al. [76]. Developed nanoMIPs were prepared by a surface imprinting of a whole viral template (phage) using the miniemulsion polymerization. The fabricated nanoMIPs enabled the selective recognition of the imprinted phage and displayed remarkable antiviral properties. Moreover, the capability of the nanoMIPs to hinder viral infections in the presence of the host cells was investigated. It was found that bacterial samples treated with nanoMIPs had in the presence of phages significantly higher growth rates. The improvement of the bacterial growth rate after 4 h of infection by phage in the presence of the nanoMIPs was 28.5%. These results represent a significant breakthrough in the field of molecular imprinting and antiviral therapy [76].

The imprinting of viruses has attracted significant attention, especially in the field of diagnostics, nevertheless, another study in the area of nanoMIP-based antiviral therapy appeared only in 2017 [77]. In that study, Li et al. introduced biocompatible and nontoxic polydopamine nanoMIPs selective for a whole virus template (i.e., bacteriophage) [77]. In contrast to the paper published by Sankarakumar et al. [76], the paper by Li et al. was enriched by a study of the effect of the environment (i.e., ionic strength, pH, viscosity, etc.) on the recognition ability of nanoMIPs. Moreover, an investigation of biocompatibility, cytotoxicity, and stability of developed imprinted particles was provided.

The first nanoMIPs selective for a mammalian virus (i.e., porcine reproductive and respiratory syndrome virus—PRRSV-1) were introduced by Graham et al. [78]. The nanoMIPs enabled specific neutralization of the viral infection in vitro within a clinically relevant incubation time (2.5 min). The specificity of nanoMIPs was investigated using the unrelated bovine viral diarrhea virus-1 and no significant cross-reactivity was observed. The data confirm the potential of nanoMIPs as stable and economical synthetic antibodies in the treatment and prevention of viral infections [78].

In 2019, a study focused on a clinically relevant human virus was published [79]. The work published by Xu et al. describes the synthesis and characterization of nanoMIPs for the recognition and blocking of a highly specific peptide motif—SWSNKS (3S). 3S is an epitope of the surface glycoprotein 41 (gp41) occurring in the human immunodeficiency virus type 1 (HIV-1). This part of gp41 is implicated in the decline of CD4+ T cells, which leads to the deterioration of the immune system function during HIV infection. Targeting and blocking the 3S

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peptide prevents subsequent cascade interactions causing the killing of CD4+ T cells. The solid-phase synthesis approach was used to prepare water-soluble nanoMIPs. Unlike the classical epitope imprinting using mainly linear epitopes, in that work, a template mimicking the three-dimensional structure of 3S in gp41 was used. It was proven that developed nanoMIPs bind the 3S motif both alone (IC_{50} of 18.7 nM) and within the gp41 protein (IC_{50} of 85.4 nM) with high affinity and selectivity. In this way, the ability of these nanoMIPs to block the function of 3S peptide in vitro was demonstrated. These findings indicate that the developed nanoMIPs may serve as therapeutics and eventually be engineered to become an immunoprotective HIV vaccine. However, the effectiveness of nanoMIPs must be confirmed in the presence of HIV-1 during infection of the host cell. Also, further cytotoxicity evaluation and pharmacokinetic, and antiviral studies in vivo are necessary. Nevertheless, this publication [79] constitutes an important breakthrough in nanomedicine and nanotherapy.

In 2020, a pandemic caused by the virus SARS-CoV-2 led to the boom in support of the research and development of virus diagnostic assays, antivirotics, and vaccines. In a very short period of time, several methods and approaches focusing on the diagnosis, treatment, and prevention of COVID-19 illness appeared [9] including the nanoMIP-based diagnostics assays and therapeutics. Parisi et al. [80] presented a research study aimed at the development of nanoMIPs capable of selective binding and inhibiting the coronavirus SARS-CoV-2 spike protein which is responsible for the coronavirus entry into host cells. NanoMIPs were prepared by the microemulsion polymerization and for the rational selection of appropriate monomers, a computational approach was used. Prepared nanoMIPs were able to recognize SARS-CoV-2 RBD (Receptor binding domain) with a detection limit of 1 ng. The specificity was verified by a dot blot method using a structural analog—the receptor-binding domain of SARS-CoV. Obtained results showed that the particles bound up to 44% of the analyte and only 4% of the competitor molecules. In that study, it was also found that the developed nanoMIPs were capable of significant inhibition of the virus replication in vitro. NanoMIPs concentration of 20 ng μL^{-1} efficiently inhibited the viral growth, with an inhibition effect of 99%. This suggests that the approach can be potentially used as a strategy to prevent infection and as a treatment for SARS-CoV-2-positive patients to inhibit viral replication in the early phase of infection. Moreover, nanoMIP-based therapeutics could be potentially used in combination with antiviral agents as a powerful multimodal system combining the ability of nanoMIPs to block the viral spike protein with the targeted delivery of a drug [80].

TABLE 2 | Overview of imprinted polymers serve as antivirotics.

Virus	Imprinted part	Type of polymerization	Functional monomer	Year	Ref.
Bacteriophage	Whole-cell imprinting	Microemulsion polymerization	Methacrylate	2013	[76]
Bacteriophage	Whole-cell imprinting	Core-shell imprinting (silica particles)	Dopamine	2017	[77]
PRRSV-1	Whole-cell imprinting	Precipitation polymerization	Acrylamide	2019	[78]
HIV-1	Epitope imprinting (epitope of gp41)	Solid phase synthesis	Methacrylate	2019	[79]
SARS-CoV-2	Protein domain imprinting (RBD domain of spike protein)	Microemulsion polymerization	Methacrylate	2020, 2021	[80, 81]

4.2 | NanoMIPs as Antidote

Another biological target can include non-pathogenic xenobiotic proteins/peptides, especially venoms. The peptides of venom usually contain between 20 and −120 residues and contain up to eight disulfide bonds that are critical for biological activity and stability [82, 83]. These biomolecules cause a disturbance in the fundamental physiological systems of the victim, which can lead to death if left untreated [84]. Currently, most antivenoms are antibody products that can disable a

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specific venom's toxins. Therefore, the preparation of synthetic antibodies—nanoMIPs selective for venom peptides/proteins is an appealing strategy.

In 2010, Hoshino et al. [7] introduced the nanoMIPs, which capture and clear a bee peptide toxin—melittin from a mouse's bloodstream. The work is exceptional not only because it was the first to point out the possibility of using nanoMIPs as an antidote, but especially because it proved for the first time that nanoMIPs can effectively work in the bloodstream of living animals. Although molecular recognition of nanoMIPs has been extensively studied in controlled settings, reports describing their application in the bloodstream of living animals are rare. The introduction of nanoMIPs into a complex biological milieu can significantly affect their performance including affinity, specificity, and function and therefore it is necessary to examine them thoroughly. In an in vivo environment, several complications can arise. The first of them is the absorption of proteins and the formation of a protein corona on the nanoMIPs surface immediately after nanoMIPs entry into the bloodstream [73]. Another issue is connected to the possible organism immunogenic response. Before the evaluation of nanoMIPs efficacy in vivo, their biocompatibility was tested. It was found that there was no significant difference in body weight between control mice and groups administered nanoMIPs and also no detectable toxicity was observed histopathologically in tissue samples from the liver, lung, or kidney 2 weeks after nanoMIPs injection. The ability of nanoMIPs to neutralize the toxicity of melittin was tested in vivo by their systemic administration following injection of the toxin while the controls did not receive nanoMIPs dose. Results showed that in mice with intravenously administered melittin 100% mortality occurred. Whereas upon intravenous infusion of nanoMIPs, a significant decrease in mortality was observed ($p = 0.030$). This indicates that nanoMIPs can recognize the specific toxin melittin and neutralize its activity in the bloodstream. Further, it was found that the melittin-nanoMIPs complexes were then cleared from the bloodstream by the mononuclear phagocytic system in the liver [85, 86].

The development of nanoMIP-based antidotes was continued by Sogo et al. [87] who described nanoMIPs capable of neutralizing the activity of CTX cytotoxins present in the venom of the Mozambican cobra. The effectiveness of nanoMIPs to inhibit the hemolytic activity of CTX peptide was studied by pig erythrocytes. It was found that nanoMIPs effectively reduce the extent of hemolysis, with the concentration of 2.5 g L^{-1} eliminating whole hemolytic activity. These results indicate that the developed synthetic antivenom has therapeutic potential.

5 | Conclusions

In the last few years, nanoMIPs have been widely studied for both inhibiting and activating enzymes, blocking receptors or their ligands, preventing viral infection, and neutralizing toxic substances. Imprinted particles showed an impressive recognition ability of target biomolecules in both model and real conditions. Based on in vivo and in vitro cytotoxicity assays, described nanoMIPs seemed to be biocompatible, but of course, depending on their composition. The most promising imprinted particles are composed of poly(N-isopropylacrylamide) and other polyacrylamides. Based on the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, results obtained using a variety of non/cancerous cell lines, poly(N-isopropylacrylamide) is not considered as significantly toxic. Moreover, polyacrylamides are commonly present in food and low concentrations do not represent a health risk. So far, imprinted particles have been studied in two model organisms specifically mice, and zebrafish embryos. It was found that nanoMIPs do not exhibit pathological toxicity to the main internal organs (liver, kidney, heart, spleen, lung) in mice or reduced viability and/or alterations of normal development of zebrafish embryos. However, many debates question the reliability of mice as a model organism for human diseases as many drugs that work well in preclinical studies in mice were proved ineffective when used in human clinical trials. Therefore, a deeper study of biodistribution and clearance in vivo is needed including the effect of their size, charge, stiffness, and immunogenicity. Even though the development of nanoMIPs for therapeutic purposes is still at the beginning, the results so far seem very promising. The developed nanoMIPs have been shown to have the potential to work as a drug itself, mimicking monoclonal antibody therapy. Although they faced many challenges at the initial stage, nanoMIPs have opened a new research avenue and exhibit great potential in cancer nanomedicine. With the progress in molecular imprinting, nanoMIPs and their superior properties will significantly advance cancer therapy.

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Data Availability Statement

The literature analysis was based on Web of Science-published publicly available articles.

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