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Design, synthesis and use of peptoids in the diagnosis and treatment of cancer

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1. ABSTRACT

Genetic variations in cancer cells are the underpinning for the development of resistance and failure of the treatment by current anticancer drugs. Thus, an ideal drug must overcome failure of treatment and prevents development of drug resistance. There are a wide variety of emerging, easy to prepare and cost effective group of drugs that are collectively called peptoids or peptidomimetics. These new set of drugs exhibit distinct features including protease resistance, are non-immunogenic, do not hinder functionality and backbone polarity, and can adopt different conformations. These drugs have shown promise as diagnostic and therapeutic tools in a wide variety of diseases. Here, we discuss the recent advancement in the design and synthesis of peptoids and use of these drugs in the diganosis and treatment of a wide number of cancers of the lung, prostate, and breast.

2. INTRODUCTION

Peptoids are protease resistant, achiral, and non-immunogenic, and without hindering functionality and backbone polarity, can adopt different conformations (1,2). Peptoids have emerged as versatile molecular tools in the field of biochemistry and biophysics and have been used in diagnosis and treatment of cancer.

Cancer is a heterogeneous disease, that exhibits disparate response to drugs, and substantial variations in long-term patient survival. Current chemo- and radiotherapies target the growth of neoplastic cells. A "perfect anti-cancer chemotherapeutic agent" still does not exist and certain drugs are ineffective in a large number of patients with cancer. Conventional chemotherapeutics exhibit limited efficacy, mainly due to a variety of side effects. Therefore, new drugs are required that can target cancer cells without any off-target effect. Recently, peptoids have received increased attention as novel anti-cancer drugs. Peptoids are peptidomimetics or peptide-like molecules, which have shown promising effects in cancer treatment. Peptoids were first synthesized in the early 1990s (3,4) and have shown to be advantageous over traditional treatments using antibodies, peptides and nanoparticles because of better bio-distribution, tumor affinity, tumor penetration, clearance from the body and lower *in vivo* degradation. Peptoids may also reduce the toxic side effects of anti-cancer drugs, and likely can reduce the cost of cancer treatment. Peptoids (up to ~50 amino acids in length) contain controlled sequence composition and diverse side chains, and usually have a peptide-based backbone and N-substituted glycines (Figure 1). Like the alpha carbon of peptides, the side chain of peptoids are placed on the nitrogen atom of the amide group. Peptoids like PMC (2,2,5,7,8-pentamethylchroman-6-sulphonyl) have protected guanidinopropyl amine monomer. These were first described by the Zuckermann group, and further modified by Annelise Barron *et al.*, to generate PMC, a mixed guanido/amino linear peptoid (5,6). However, their poor solubility resulted in low coupling efficiency and the extended cleavage time essential for the PMC group led to acid-induced degradation of the mixed peptoids. Sub-monomer peptoid synthesis uses conventional solid-phase approach (on resin beads) (Figure 2). Introduction of both lysine-type and arginine-type monomers within the same sequence has modeled a new strategy for synthesis of effective peptoids (7). Also by using the protocol developed by Jonathan Rothbard *et al*, new polyarginine-type peptoids have been synthesized and

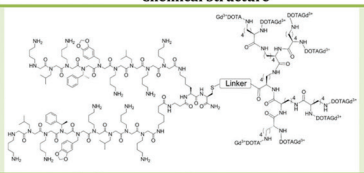
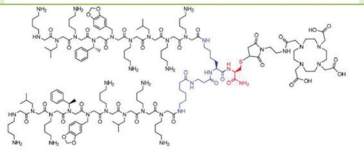
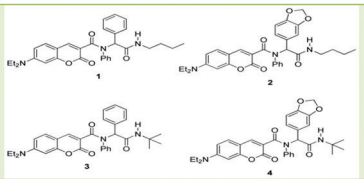
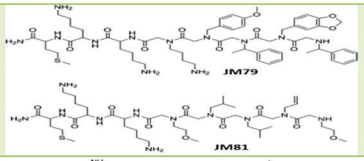
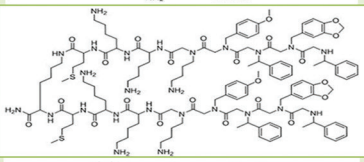
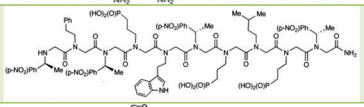
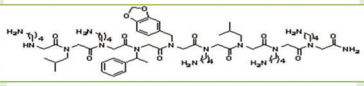
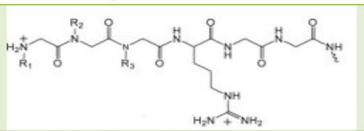
Name	Chemical Structure	Usage	Reference
Peptoid- $(Gd)_8$-dendron		VEGFR2 binding diagnostic peptoid used in Magnetic Resonance Imaging of cancerous growth	18
^{64}Cu-DOTA-GU40C4		VEGFR binding diagnostic peptoid used in positron emission tomography (PET) scanning of cancerous growth	19,20
Fluorescent Peptoid (1-4)		Diagnostic flurescent peptoids specific for mitochondrial staining of cancerous cells	21
FRET-based fluorogenic tetrapeptide tagged with C-terminus "lysine-like" nonaresidue peptoid		Cancer cell permeable cationic peptoids having FRET-based fluorogenic substrates against caspase-3 used for cancer diagnosis	23
JM79 and JM 81		Therapeutic peptoids having anticancer activity against lung cancer cells	30
PPS1D1		Therapeutic peptoid enhances the efficacy of docetaxel and also targeting Phosphatidylserine of cancer cells	33
Phosphonate containing peptoid with p-Nitrophenyl sidechains		Peptoids helping in cancer therapy by binding with HDM2 and inhibiting HDM2-p53 intercation	36
GU40C		peptoid-based therapeutics for antiangiogenic therapy by inhibiting VEGFR	39
Library of 7680 N-substituted oligoglycines		Small therapeutic peptoids binds with miRNA precursor involved in cancer progression	44

Figure 1. Chemical composition and strcutrue of various peptodids.

to improve bio-molecular recognition of targets, an organic moiety is added to the backbone structure (8). Furthermore, new polymeric peptoids that spontaneously attain distinct supramolecular structures are effectively being used for the synthesis of designer drugs for the treatment of different diseases including cancer.

3. RECENT ADVANCEMENTS IN PEPTOID DESIGN AND SYNTHESIS

Variations in the secondary, tertiary, and random structures of peptide-like oligomers were designed by several groups for generating different landscapes of peptoid structure. These modifications conferred disease-specificity to the peptoids. Additionally, the creation of effective peptoids have inspired and guided protein expression, protein-protein interaction, and protein folding. Recently, Robertson *et al*, have successfully designed peptoid nanosheets which can target specific proteins and serve distinct functionalities (9). Computational tools have aided in the generation of efficient non-biological synthetic heteropolymeric peptoids (10,11). Every design utilizes the accurate force fields to understand the

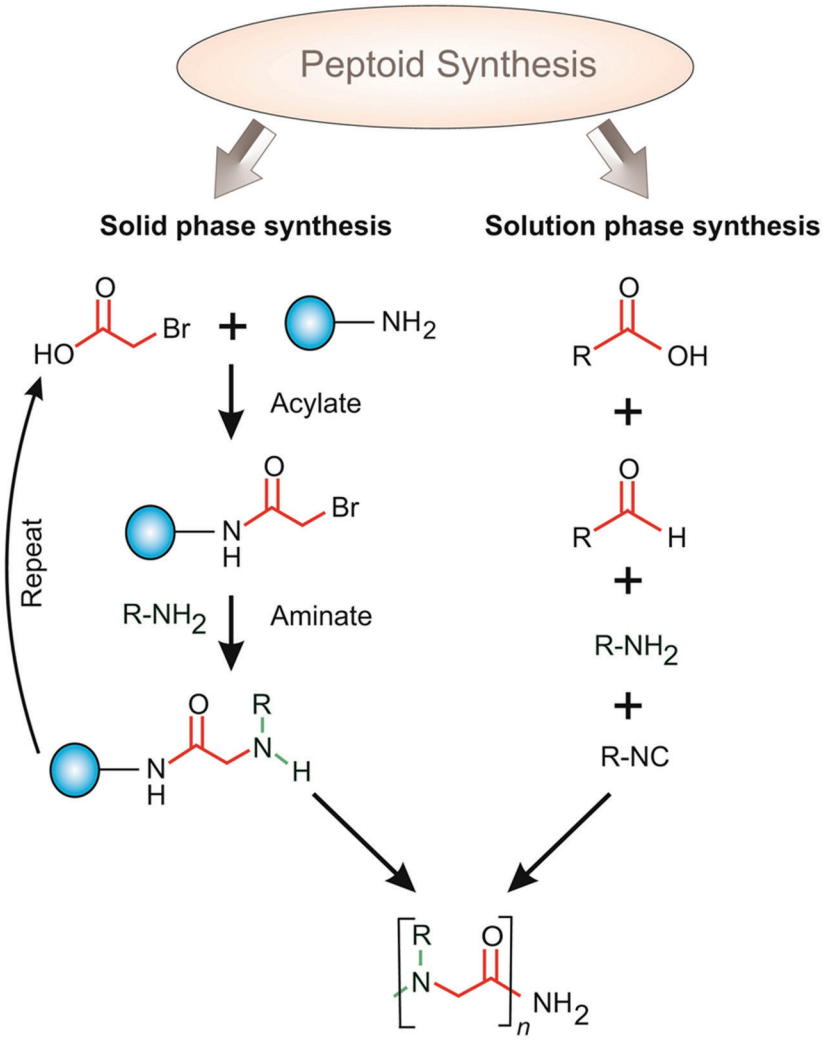


Figure 2. Schematic diagram of solid phase and solution phase peptoid synthesis.

complex peptoid architectures. Properly designed peptoids are efficiently synthesized using a solid-phase and solution-phase approach. In solid-phase method, synthesis of polypeptoids up to ~50 units in length has shown excellent yield, and has allowed for incorporation of various side chains with controlled sequence composition (4,12). This method is further improved by successful incorporation of amine derivatives of heterocycles such as histamine, pyridine, and tryptamine (13). On the other hand, in solution-phase synthesis, bromoacetylbromide is used for acylation reaction with the N-terminus of the peptoid chain. In most cases, several cycles of filtration, evaporation, and chromatography have been applied to produce reasonably large quantities of peptoids (14). Advancement of solution-phase synthesis method is achieved by "Ugi four components reaction (4-CR)" in which a primary amine, a carboxylic acid, an isocyanide, and an oxo compound react to form a dipeptoid backbone (15). This process generates macrocyclically diversified peptoid polymers of stable secondary structures to mimic native protein. As a result, complex structures consisting of long chains within a single type of polymer have been successfully developed to create helix bundles comprising of hydrophobic cores (16). Binding of high affinity zinc into the peptoid two-helix bundles has also been introduced for improvement in peptoidal mimicry of biological proteins (17). Therefore, innovative methods, computational designs, and synthetic approaches, have revolutionized the peptoid chemistry and have led to the development of more complicated protein-like structures targeting specific proteins.

4. BIOLOGICAL APPLICATIONS OF PEPTOIDS IN CANCER

4.1. Application of peptoids in cancer diagnosis

Peptoids have been used as a tool in cancer imaging and diagnosis. For example, (MRI)

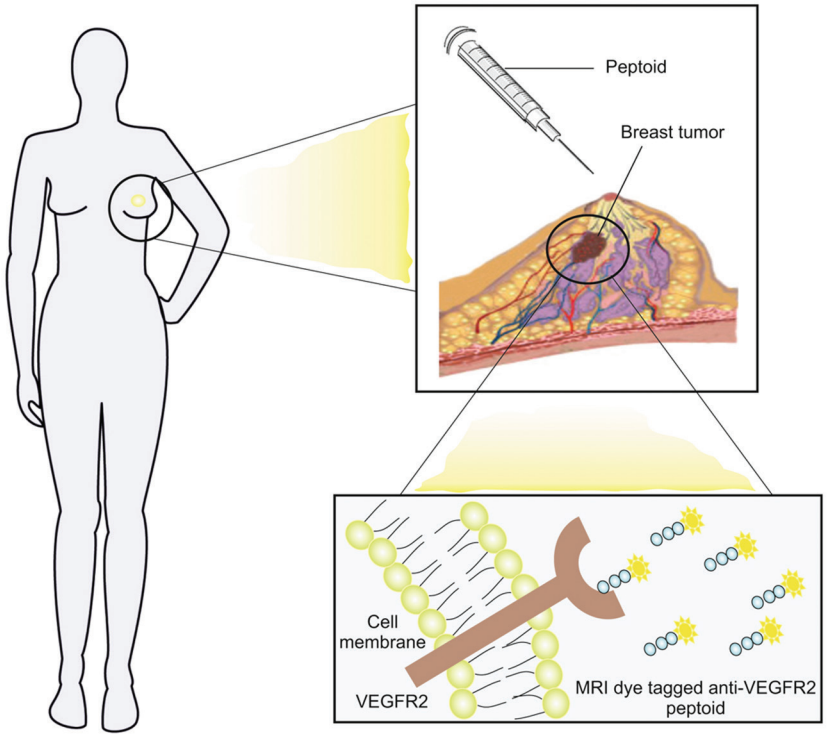


Figure 3. Use of peptoids in cancer diagnosis. Figure represents one of the possible usage of MRI dye tagged anti-VEGFR2 peptoids for breast cancer diagnosis.

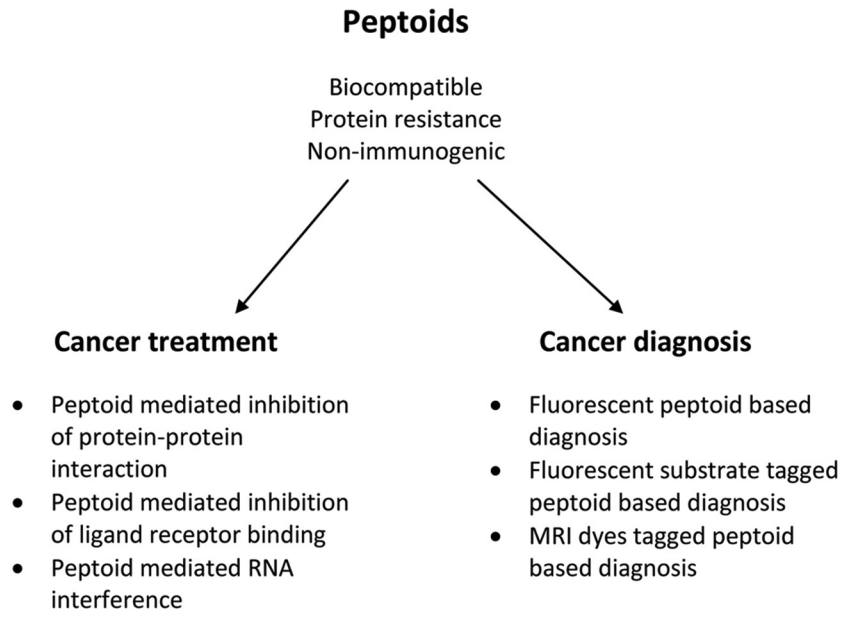


Figure 4. Different biological applications of anti-cancer peptoids.

[Gd(III)-DOTA)8 dendron] added to the VEGFR2 binding peptoid has been used in Magnetic Resonance Imaging of breast cancer (Figure 3) (18). Cai *et al.,* 2011, reported the use of a peptoid-based PET imaging of VEGFR overexpression in cancer diagnosis (19). In another study, a peptoid-based positron emission tomography (PET) tracer was used for imaging of VEGFR expression in prostate cancer (19,20). Passos

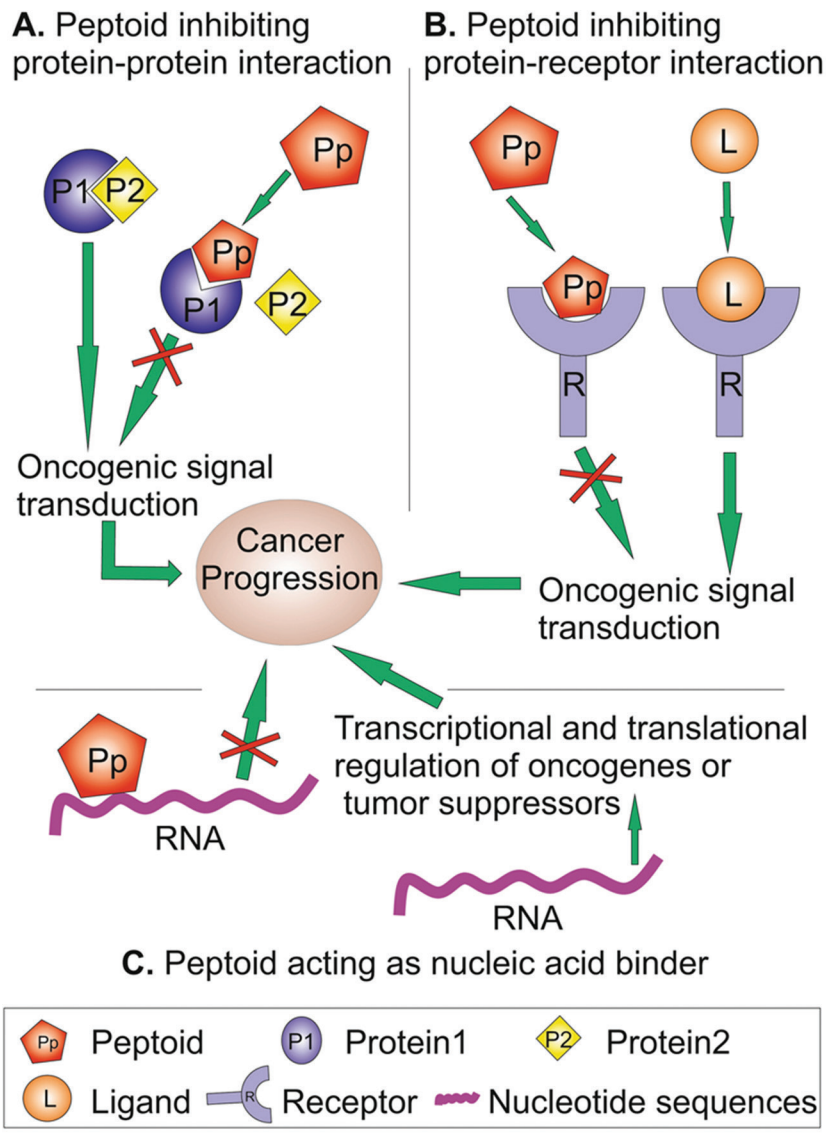


Figure 5. Use of peptoids in cancer therapy. A. Peptoid mediated inhibition of protein–protein interaction such as binding of peptoid with HDM2 results in inhibition of HDM2-p53 interaction that plays a crucial role in cancer progression. B. Peptoid mediated inhibition of ligand and receptor interaction such as targeting VEGF signaling by anti-VEGFR2 peptoid to halt tumor progression. C. Binding of peptoid to a nucleotide sequence including RNAs such as precursor of miR-21, to prevent tumor progression.

et al, described the synthesis of fluorophore labeled peptoids using the Ugi multicomponent reaction and demonstrated internalization and localization of fluorescent peptoids in breast cancer cells (4-CR) (21). Because caspase-3 is increased in cancer, *in situ* monitoring of caspase-3 activity has been proposed as a means for cancer diagnosis (22). For detection and quantification of caspase-3 activity, Pérez-López *et al* desribed the use of FRET-based fluorogenic substrates against caspase-3 conjugated to the cell permeable cationic peptoids (23). Together, such studies establish the role of fluorescent peptoids and fluorescent molecule tagged peptoids as a prominent and promising approach in cancer diagnosis.

4.2. Application of peptoids in cancer treatment

Peptoids exhibit high bioactivity as protein mimics, which, in turn, can reduce the dose of anti-oncogenic antibodies required in the treatment of cancer. Additionally, peptoids can also replace the use of small molecules developed for cancer treatment (Figures 4 and 5). Moreover, generation of large combinatorial libraries of such peptoids provide the options of high throughput screening and effective selection of the anti-oncogenic peptoids. Peptoid libraries are usually generated by the “split-pool” approach, which direct the development of ‘one-bead one compound’ libraries with huge diversity (24-27). Thomas Kodadek *et al* showed that cells are permeable to most peptoids.

In their work, the group applied peptoids to the biological systems and proved their therapeutic efficacy (28).

Many peptoids have been found to be effective, even at low doses, in the treatment of a broad range of cancer cell lines and multidrug resistant cancer cells. Cationic, amphipathic peptoids were shown to have a significant inhibitory effect on tumor growth in a human breast cancer xenotransplantation model (29). In addition to these studies, homodimers, homotrimers, and heterodimers of peptoids have been shown to interact with biological targets present on the surface of the lung cancer cells (30). Lee *et al*, used a peptide-peptoid hybrid in the treatment of prostate cancer cells (31). Peptide–peptoid hybrid PPS1D1 has been shown to have anti-lung cancer activity (32). Desai *et al*, showed that the peptoid, PPS1D1, enhanced the efficacy of docetaxel in mice bearing H460 lung cancer xenografts (33).

Among the cell cycle regulatory proteins, the loss of action of the tumor suppressor protein, p53, is involved in oncogenic progression. Loss of regulatory function is largely due to mutations and the inactivation of the cell cycle check points controlled by wild type p53 (34,35). Also, in cancer cells, overexpressed Human Double Minute 2 (HDM2) factor binds to p53 protein and inactivates its function leading to tumor aggressiveness and drug resistance. Therefore, Toshiaki Hara *et al* have used for targeting p53 signaling network with peptoid as a treatment strategy by designing and optimizing oligomeric peptoids that target HDM2-p53 interaction (36). AKT is one of the most commonly activated signaling pathways in various types of human cancer (37). AKT is a Ser/Thr kinase which transduces signals from growth factors to downstream targets that control tumor development. Peptoids have been shown to be highly selective due to their extensive interactions with the specific substrate binding site (38). Peptoids have been also used for inhibition of angiogenesis by targeting VEGF receptor-2 (VEGFR2) which causes reduced angiogenesis and hence tumor development (39). Peptoid-based cap groups (Cal27 CisR) that inhibit HDAC6 activity have shown enhanced chemosensitizing properties and led to reversion of the cisplatin resistance in squamous carcinoma cells (40).

Targeting RNA with peptoids is a novel strategy in cancer treatment (41). Some of such peptoids have been identified by high-throughput screening of peptoid library. Peptoid scaffolds with RNA binding moieties have been used in microarrays, to screen, profile, and quantify their interactions with RNA-binding molecules (42,43). For example, peptoid microarrays are used to identify specific ligand for the RNA hairpin precursor of miR-21, a microRNA, which is up-regulated in different forms of solid tumors (44).

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