Synthesis, Characterization, and DNA Binding Profile of a Macrocyclic β -Sheet Analogue of ARC ProteinAzzurra Stefanucci,[†] Jesús Mosquera,[‡] Eugenio Vázquez,[‡] José L. Mascareñas,[‡] Ettore Novellino,[§] and Adriano Mollica^{*,||}[†]Dipartimento di Chimica, Sapienza, Università di Roma, P.le A. Moro 5, 00187 Rome, Italy[‡]Centro Singular de Investigación en Química Biolóxica e Materiais Moleculares (CIQUS), Departamento de Química Orgánica, Universidade de Santiago de Compostela, 15782 Santiago de Compostela, Spain[§]Dipartimento di Farmacia, Università di Napoli "Federico II", Via D. Montesano, 49, 80131 Naples, Italy^{||}Dipartimento di Farmacia, Università di Chieti-Pescara "G. d'Annunzio", Via dei Vestini 31, 66100 Chieti, Italy

S Supporting Information

ABSTRACT: ARC repressor (apoptosis repressor with caspase recruitment domain) is a protein which binds selectively to a specific sequence of DNA. In humans, ARC is primarily expressed in striated muscle tissue, which normally does not undergo rapid cell turnover. This suggests that ARC may play a protective role in the prevention against Duchenne Muscular Dystrophy and several types of tumors. In this Letter we report the synthesis, characterization, and conformational analysis of a β -sheet ARC repressor mimetic, based on the amino acid sequence of the β -sheet domain in the ARC protein. The ability of this β -sheet macrocycle to bind to double-stranded DNA was also evaluated using spectroscopic methods. Our data show that the synthetic peptide has a defined conformation and is able to bind DNA with reasonable affinity. These initial results lay the groundwork for the design of novel β -sheets folded peptides as valuable substitutes of transcription factor proteins in drug therapy.

KEYWORDS: ARC repressor, DNA recognition, major groove, transcription factors, β -sheet macrocycles, solid phase peptide synthesis

Gene expression is finely regulated by transcription factors (TFs). The development of synthetic entities able to mimic their ds-DNA (double stranded DNA) recognition properties could potentially cure diseases caused by alterations in their activity, such as cancer^{1–3} and Duchenne Muscular Dystrophy.⁴ ARC repressor is a transcription factor abundantly expressed in the striatum human muscle and cardiac tissue that selectively interacts with the caspase recruitment domain (CARD) of caspase-2 and caspase-8, thus inhibiting cell death caused by hypoxia and hydrogen peroxidase-mediated cells H9c2, protecting cardiac muscle from postischemic cardiomyopathy.⁵ Its abundance in long life cells suggests a protective action on the muscular fibers involved in apoptotic processes caused by mechanical stress and oxidative damage.⁵ The ARC repressor protein belongs to a superfamily of TFs called RHH (ribbon-helix-helix), on the basis of the order assumed by the elements that constitute its secondary structure

(Figures 1a,1b).⁶ The crystallized complex ARC–DNA (PDB: 1PAR) reveals a tight recognition of the β -sheet (ribbon) present in the small domain of ARC to specific DNA sequences (Figure 1c).⁷ The functional unit of ARC is a dimer referred to as RHH2, characterized by a double folded symmetry and capable of binding to specific DNA sequences, including the TAGA box.^{6,7} The two identical β -strands present in the amino terminal ends of each monomer form an antiparallel β -sheet (Figure 1b), which binds to the major groove of the DNA.⁸ During the recognition process the N to C direction of each strand coincides with the 3 to 5 direction of the near strand and the local β -sheet axis is bulged toward the DNA, generating the characteristic convex curvature of a two-strand that fits in the

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Figure 1. (a) Structure of the ARC repressor dimer in complex with its DNA target site (PDB: 1PAR). One dimer is shown in purple; the other highlights each of the monomers in green and light-blue; (b) Secondary structure of an ARC dimer. (c) Detail of the β -sheet fragment that contacts the DNA (PDB: 1PAR). The figure has been prepared by using Maestro 9.2 (Schrodinger).¹⁰ (d) Relevant CH-CH distances in Å of the β -sheet ARC repressor (PDB: 1ARR).

major groove (Figure 1).⁹ The β -sheet structure interacts with six consecutive base pairs by Gln, Arg, Asn side chains, establishing contacts with key sequence-specific nucleotide bases by an intensive set of hydrogen bonds.⁷ Sequence-specific binding between β -strand residues to the major groove of DNA is an exclusive feature of the RHH superfamily and distinguishes it from the ubiquitous HTH (helix-turn-helix), that binds the DNA by an α -helix.⁶ During the recognition process, the β -sheet domain undergoes an induced conformational adjustment: in the free protein, the side chains of both Phe present in each β -strands are buried in the hydrophobic core,¹¹ but when the protein binds to the DNA, these chains shift outward to fit between the oxygens of the phosphate groups, while the C $^{\alpha}$ residues of Gln and Phe move about 1.0–1.7 Å to partially fill the cavity generated by the repositioning of phenyl rings.¹² The occupation of the site operator on the DNA by ARC is governed by fine monomer–dimer equilibrium and by a cooperative oligomerization of RHH dimers when they bind to the DNA adjacent subsite operators.⁶ The analysis of the literature reveals that many efforts have been devoted to developing artificial DNA binding peptides mimicking natural

TFs, but most of them rely on the use of β -helical motifs of b-ZIP or Zn-Finger proteins.^{13,14}

In contrast, the DNA binding motifs based on a β -sheet like that of ARC protein have not yet been explored.

Considering the recent growing interest in the ARC protein as a therapeutic target in several human diseases,¹⁵ and the identification of the 3D structure of ARC protein in solution (PDB: 1ARR, 1ARQ)^{16,17} and in complex with DNA (PDB: 1PAR),⁷ here we describe the first attempt to develop a mimetic β -sheet peptide designed to fold into a well-defined β -sheet conformation, on the basis of the structural moiety found in the native ARC protein.

The design of the first macrocyclic β -sheet ARC repressor mimetic is based on the sequence of the β -sheet portion of the native ARC protein PQFNLRWP//PQFNLRWP# (Figure 1), whereas the four prolines have been replaced by two Orn δ linkers in order to close the cycle (see product 4).

The key amino acids of each β -strand have been preserved to ensure the sequence-specific interactions with the nucleotide bases and the correct positioning of the β -sheet in the major groove.⁷ Two residues of Lys were incorporated into the native sequence to enhance the water solubility.

The Orn residues have been chosen in order to fold the molecule portions into β -hairpin turns and therefore stabilize the β -sheet organization,¹⁸ keeping the molecular symmetry and positioning necessary for the recognition of an extensive sequence of DNA bases. Macrocyclic β -sheet ARC analogue 4 was synthesized by solid phase peptide synthesis (SPPS), starting from preparing the full protected linear peptide 2 carried out on 2-chlorotrityl chloride resin by standard automated Fmoc SPPS (Scheme 1).¹⁹ Macrocyclization of peptide 2 was performed on a solution of 0.5 mM peptide concentration with 4 equiv of HCTU and 8 equiv of DIPEA in DMF over 64 h. The so obtained crude product 3 was deprotected with TFA/TIPS/H₂O 18/1/1 and lyophilized after dissolution in water, to facilitate cleavage of tryptophan carbamate (Trp-COOH) groups. The resulting crude peptide 4 was then purified by RP-HPLC and characterized by mass spectra. Lyophilization of the pure fractions yielded the macrocyclic peptide 4 in 12% overall yield (0.030 g), based on theoretic loading of the resin with Boc-Orn(Fmoc)-OH (for detailed experimental procedure, see Supporting Information). Assignments of the signals were done by ¹H NMR (Table S1) and 2D TOCSY NMR, and for the connectivity, sequential NOEs were used (see Supporting Information). In model 4 a strong NOE between CH $^{\alpha}$ Trp and CH $^{\alpha}$ Phe was found (Figure S1, Supporting Information) with the absence of the CH $^{\alpha}$ Arg-Gln NOE, which is a key feature of the β -sheet present in the ARC repressor structure. This finding, together with the sequential NOEs found (Table S2, Supporting Information), strongly suggests a quite different conformation of the model synthesized. Centered on the two Leu and Phe residues may be present a large hydrophobic cluster. The structure is also stabilized by π – π interaction between the Trp aromatic rings and lipophilic interaction between Phe aromatic rings and Leu side chains. The folding of product 4 was also studied by Circular Dichroism (CD) in 10 mM aqueous phosphate buffer (pH 7.5). Under these conditions, product 4 showed an apparently unusual CD spectrum with two intense positive bands at 195 and 225 nm (Figure S4, Supporting Information). The theoretical CD of an antiparallel β -sheet should consist of one positive band around 195 nm and one negative band around 218 nm. However, the intense exciton-

Scheme 1. Synthesis of the Final Product 4 as TFA Salt

Figure 2. DNA binding of peptide 4 studied by tryptophan fluorescence spectroscopy. (a) Fluorescence emission spectrum of a 0.5 μ M solution of peptide 4 in HEPES buffer 10 mM pH 6.5, 100 mM NaCl after addition of 0, 0.5, and 1 equiv of **DNA1**. (b) Fluorescence emission spectrum of 0.5 μ M solution of peptide 4 in HEPES buffer 10 mM pH 6.5, 100 mM NaCl after addition of 0, 0.5, and 1 equiv of **DNA2**. Oligonucleotide hairpin sequence (binding site in *italics*): **DNA1**: 5'-GCGAG TAGA GC TTTT GC TCTA CTCGC-3'; **DNA2**: 5'-GCGAG CACA GC TTTT GC TGTG CTCGC-3'.

coupled band between 215 and 229 nm is consistent with the interaction between indole rings, in agreement with previous NMR studies.²⁰ Considering this aspect, the CD spectrum of final product 4 fits with a β -sheet structure. DNA binding properties have been studied through fluorescence spectroscopy. Since peptide 4 contains two tryptophan amino acids, tryptophan fluorescence spectroscopy was applied to check the interaction with DNA. The tryptophan fluorescence is strongly influenced by the local environment; then its fluorescence

quenching has been used to investigate protein conformational changes²¹ and protein–DNA interaction.²² In the case of DNA interaction, the quenching is mainly due to the electron transfer from indole ring to DNA bases. As expected, addition of a solution of ds-oligonucleotide **DNA1**, which contains the consensus target for this peptide (TAGA), over a solution of peptide 4, induces a decrease in the tryptophan signal (Figure 2a). Surprisingly, the drop of the signal almost stops completely with only 0.5 equiv of the DNA. This can be explained

considering that peptide 4 might interact with the DNA in several positions simultaneously, when the concentration of DNA is lower than that of the peptide. In order to further evaluate the sequence specificity of peptide 4, the experiment was repeated by using a DNA with a double mutation in the consensus sequence (**DNA2**) (Figure 2b). Interestingly, the peptide showed exactly a close behavior with both **DNA1** and **DNA2** consensus sequences. Finally, the ethidium bromide displacement method was used to corroborate the DNA interaction, analyzing the loss of fluorescence derived from the displacement of ethidium bromide by another agent that interacts with the DNA (Figures S5, S6, SI).²³ This experiment also confirmed the interaction of the peptide with ds-DNA, and as in the case of tryptophan quenching, peptide 4 showed the same interaction with **DNA1** and **DNA2**. Considering that peptide 4 displaces two ethidium bromide molecules after the DNA binding, we could calculate an approximate dissociation constant of 2.1 μM at 20 $^{\circ}\text{C}$ (for experimental details, see SI).²⁴ Despite the fact that the peptide does not exhibit the expected specificity, it is able to displace the ethidium bromide; this implies that it established not only electrostatic interactions with the DNA phosphates, but is docked in one of DNA grooves, making contacts with bases. Isolated transcription factor fragments typically display very low affinity for their cognate DNA sites. For example, monomeric GCN4 basic regions show a K_D of $\approx 20 \mu\text{M}$ for their target AP1 or ATF/CREB half sites,²⁵ and therefore the measured $\approx 2 \mu\text{M}$ binding constant shows that the designed macrocycle is capable of establishing efficient contacts with its target DNA, with affinity is in the same order of that showed by autonomous DNA-binding domains, such as the AT-Hook ($K_D \approx 6 \mu\text{M}$),²⁶ or other small organic DNA binding agents, such as propamidine, DAPI, or dystamicin.^{27–29} Conceiving molecules characterized by β -sheet-like structures to address long DNA sequences is a novel and extremely challenging task, considering that the majority of proteins binding DNA and their synthetic analogues are α -helix structures capable of binding simultaneously with both the major and minor grooves of the DNA duplex.^{30,31} A great amount of literature is focused on the study of ARC repressor factor as a promising target in transcription therapy.^{14,32–37} In this paper we reported the first attempt to synthesize a β -sheet like macrocycle as ARC repressor analogue, in order to obtain a handle biomimetic peptide recognizing specific binding sequence of DNA. The peptide showed high affinity interaction with ds-DNA but low sequence selectivity. Based on the above observations, in order to obtain a β -sheet type peptide capable of binding to the DNA major groove, one option could consist of trying to avoid the hydrophobic collapse replacing the Leu residues with Ser, which may reduce the hydrophobic clustering and permit the desired conformation; furthermore, the substitution of the δ -linked ornithine with D-Pro-Gly sequence may also give the desired turn.³⁸ Further work is under way to improve the design process, which involves all the necessary modifications in the amino acids sequence, also tethering to minor groove binders could be a fruitful future perspective.

EXPERIMENTAL PROCEDURES

Chemicals. HPLC grade solvents were purchased from Fisher Scientific (Pittsburgh, PA); methylene chloride was further purified by passage through a column of alumina under argon. 2-Chlorotriptyl chloride resin, HCTU, and all Fmoc-protected amino acids were purchased from Novabiochem (Massachusetts, USA); biochemical

grade trifluoroacetic acid for HPLC was acquired from Acros Organics, and standard grade trifluoroacetic acid for deprotection of peptides was purchased from Fischer Scientific (Pittsburgh, PA). D_2O and $\text{DMSO}-d_6$ were acquired from Cambridge Isotopes; all other reagents were from Aldrich Chemical Co.

HPLC and Mass Spectra. Analytical reverse phase HPLC (RP-HPLC) was performed on a Beckman (Agilent Zorbax 80 SB C18 column, $50 \times 4.6 \text{ mm}$; solvent A: $\text{H}_2\text{O}/0.1\% \text{ TFA}$, solvent B: $\text{CH}_3\text{CN}/0.085\% \text{ TFA}$). A $21.2 \times 250 \text{ mm}$ Zorbax SB-C18 PrepHT ($7\text{-}\mu\text{m}$ particle size) column from Agilent on a Rainin Dynamax system was used for preparative HPLC (flow rate 10 mL/min). UV detection (254 nm) was chosen for analytical and preparative HPLC; water and acetonitrile containing 0.1% biochemical grade TFA were used as solvents. TOF-MS ESI was used to analyze all final products and reaction intermediates.

NMR Spectroscopy. Five mg of pure analogue 4 were used for the preparation of NMR samples in PP 535 NMR tubes in 0.5 mL of D_2O (2.8 mM concentration), and another 5 mg of pure analogue 4 were used for the preparation of NMR samples in PP 535 NMR tubes in 0.5 mL of D_2O 10% (0.05 mL)/ H_2O 90% (0.45 mL) in 2.8 mM concentration; 5 mg of pure analogue 4 was used for the preparation of NMR samples in Shigem tubes using 0.302 mL of D_2O (4.67 mM concentration) and another 4 mg for the preparation of NMR samples in Shigem tubes in 0.302 mL of D_2O 10% (0.03 mL)/ H_2O 90% (0.27 mL) in 3.73 mM concentration. These spectra were performed in 500 MHz Cryo Bruker and 800 MHz Varian UnityInova.

Circular Dichroism (CD) and Fluorescence measurements. CD spectra were obtained with Jasco-715 coupled with a thermostat Nestlab RTE-11, using an acquisition range: 250–190 nm; bandwidth: 2.0 nm; resolution: 0.2 nm; accumulation: 3 scans; sensitivity: 10 mdeg; response time: 0.25 s, speed: 100 nm/min. CD measurements were performed in a 2 mm cell at 20 $^{\circ}\text{C}$. Product 4 (125 μM) was dissolved in 10 mM phosphate buffer (pH 7.5) and 100 mM of NaCl.

Tryptophan fluorescence and ethidium bromide displacement spectra were obtained with a Jobin-Yvon Fluoromax-3 (DataMax 2.20) coupled to a Wavelength Electronics LFI-3751 temperature controller, as follows: increment: 1.0 nm; integration time: 0.1 s; excitation slit width: 4.0 nm; emission slit width: 6.0 nm at 20 $^{\circ}\text{C}$. In the case of tryptophan fluorescence, the excitation wavelength applied was 295 nm and the emission spectra were acquired from 305 to 450 nm. For ethidium bromide, the excitation wavelength applied was 540 nm and the emission spectra were acquired from 565 to 650 nm.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.5b00363.

SPPS protocol, analytical data, conformational analysis, and binding studies of peptide 4 (PDF)

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Notes

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REFERENCES

- (1) Darnell, J. E. Transcription factors as targets for cancer therapy. *Nat. Rev. Cancer* **2002**, *2*, 740–749.
- (2) Look, A. T. Oncogenic transcription factors in the human acute leukemias. *Science* **1997**, *278*, 1059–1064.
- (3) Vogt, P. K. Jun, the oncoprotein. *Oncogene* **2001**, *20*, 2365–2377.
- (4) Abmayr, S.; Crawford, R. W.; Chamberlain, J. S. Characterization of ARC, apoptosis repressor interacting with CARD, in normal and dystrophin-deficient skeletal muscle. *Hum. mol. genet.* **2004**, *13*, 213–221.
- (5) Koseki, T.; Inohara, N.; Chen, S.; Núñez, G. ARC, an inhibitor of apoptosis expressed in skeletal muscle and heart that interacts selectively with caspases. *Proc. Natl. Acad. Sci. U. S. A.* **1998**, *95*, 5156–5160.
- (6) Schreiter, E. R.; Drennan, C. L. Ribbon-helix-helix transcription factors: variations on a theme. *Nat. Rev. Microbiol.* **2007**, *5*, 710–720.
- (7) Raumann, B. E.; Rould, M. A.; Pabo, C. O.; Sauer, R. T. DNA recognition by beta-sheets in the Arc repressor-operator crystal structure. *Nature* **1994**, *367*, 754–757.
- (8) Tatenno, M.; Yamasaki, K.; Amano, N.; Kakinuma, J.; Koike, H.; Allen, M. D.; Suzuki, M. Dna recognition by β -sheets. *Biopolymers* **1997**, *44*, 335–359.
- (9) Cheng, P. N.; Pham, J. D.; Nowick, J. S. The supramolecular chemistry of β -sheets. *J. Am. Chem. Soc.* **2013**, *135*, 5477–5492.
- (10) *Schrodinger Suite 2012*; Schrodinger, LLC: New York, NY (USA), 2012.
- (11) Song, W.; Guo, J. T. Investigation of arc repressor DNA-binding specificity by comparative molecular dynamics simulations. *J. Biomol. Struct. Dyn.* **2015**, *33*, 2083–2093.
- (12) Dostál, L.; Misselwitz, R.; Welfle, H. Arc repressor-operator DNA interactions and contribution of Phe10 to binding specificity. *Biochemistry* **2005**, *44*, 8387–8396.
- (13) Pazos, E.; Mosquera, J.; Vázquez, M. E.; Mascareñas, J. L. DNA recognition by synthetic constructs. *ChemBioChem* **2011**, *12*, 1958–1973.
- (14) Vázquez, M. E.; Caamaño, A. M.; Mascareñas, J. L. From transcription factors to designed sequence-specific DNA-binding peptides. *Chem. Soc. Rev.* **2003**, *32*, 338–349.
- (15) Kung, G.; Dai, P.; Deng, L.; Kitsis, R. N. A novel role for the apoptosis inhibitor ARC in suppressing TNF α -induced regulated necrosis. *Cell Death Differ.* **2014**, *21*, 634–644.
- (16) Breg, J. N.; van Opheusden, J. H.; Burgering, M. J.; Boelens, R.; Kaptein, R. Structure of Arc repressor in solution: evidence for a family of beta-sheet DNA-binding proteins. *Nature* **1990**, *346*, 586–589.
- (17) Bonvin, A. M.; Vis, H.; Breg, J. N.; Burgering, M. J.; Boelens, R.; Kaptein, R. Nuclear magnetic resonance solution structure of the Arc repressor using relaxation matrix calculations. *J. Mol. Biol.* **1994**, *236*, 328–341.
- (18) Nowick, J. S.; Lam, K. S.; Khasanova, T. V.; Kemnitzer, W. E.; Maitra, S.; Mee, H. T.; Liu, R. An unnatural amino acid that induces beta-sheet folding and interaction in peptides. *J. Am. Chem. Soc.* **2002**, *124*, 4972–4973.
- (19) Khakshoor, O.; Demeler, B.; Nowick, J. S. Macrocyclic β -sheet peptides that mimic protein quaternary structure through intermolecular beta-sheet interactions. *J. Am. Chem. Soc.* **2007**, *129*, 5558–5569.
- (20) Tape, C. J.; Willems, S. H.; Dombernowsky, S. L.; Stanley, P. L.; Fogarasi, M.; Ouwehand, W.; McCafferty, J.; Murphy, G. Cross-domain inhibition of TACE ectodomain. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108*, 5578–5583.
- (21) Ghisaidoobe, A. B.; Chung, S. J. Intrinsic tryptophan fluorescence in the detection and analysis of proteins: a focus on Förster resonance energy transfer techniques. *Int. J. Mol. Sci.* **2014**, *15*, 22518–22538.
- (22) Peviani, C.; Hillen, W.; Ettner, N.; Lami, H.; Doglia, S. M.; Piémont, E.; Ellouze, C.; Chabbert, M. Spectroscopic investigation of Tet repressor tryptophan-43 upon specific and nonspecific DNA binding. *Biochemistry* **1995**, *34*, 13007–13015.
- (23) Boger, D. L.; Fink, B. E.; Brunette, S. R.; Tse, W. C.; Hedrick, M. P. A simple, high-resolution method for establishing DNA binding affinity and sequence selectivity. *J. Am. Chem. Soc.* **2001**, *123*, 5878–5891.
- (24) Kuzmic, P. Program DYNAFIT for the Analysis of Enzyme Kinetic Data: Application to HIV Proteinase. *Anal. Biochem.* **1996**, *237*, 260–273.
- (25) Wang, X.; Cao, W.; Cao, A.; Lai, L. Thermodynamic characterization of the folding coupled DNA binding by the monomeric transcription activator GCN4 peptide. *Biophys. J.* **2003**, *84*, 1867–1875.
- (26) Dragan, A. I.; Liggins, J. R.; Crane-Robinson, C.; Privalov, P. L. The energetics of specific binding at AT-hooks from HMGA1 to target DNA. *J. Mol. Biol.* **2003**, *327*, 393–411.
- (27) Sánchez, M. I.; Vázquez, O.; Martínez-Costas, J.; Vázquez, M. E.; Mascareñas, J. L. Straightforward access to bisbenzamidine DNA binders and their use as versatile adaptors for DNA-promoted processes. *Chem. Sci.* **2012**, *3*, 2383–2387.
- (28) Wilson, W. D.; Tanious, F. A.; Barton, H. J.; Jones, R. L.; Fox, K.; Wydra, R. L.; Strekowski, L. DNA sequence dependent binding modes of 4',6-diamidino-2-phenylindole (DAPI). *Biochemistry* **1990**, *29*, 8452–8461.
- (29) Chen, F. M.; Sha, F. Circular dichroic and kinetic differentiation of DNA binding modes of distamycin. *Biochemistry* **1998**, *37*, 11143–11151.
- (30) Blanco, J. B.; Vázquez, M. E.; Martínez-Costas, J.; Castedo, L.; Mascareñas, J. L. A synthetic miniprotein that binds specific DNA sequences by contracting both the major and the minor groove. *Chem. Biol.* **2003**, *10*, 713–722.
- (31) Vázquez, O.; Vázquez, M. E.; Blanco, J. B.; Castedo, L.; Mascareñas, J. L. Specific DNA recognition by a synthetic, monomeric Cys2His2 zinc-finger peptide conjugated to a minor-groove binder. *Angew. Chem., Int. Ed.* **2007**, *46*, 6886–6890.
- (32) Wu, P.; Tang, Y.; He, J.; Qi, L.; Jiang, W.; Zhao, S. ARC is highly expressed in nasopharyngeal carcinoma and confers X-radiation and cisplatin resistance. *Oncol. Rep.* **2013**, *30*, 1807–1813.
- (33) An, J.; Mehrhof, F.; Harms, C.; Lättig-Tünnemann, G.; Lee, S. L.; Endres, M.; Li, M.; Sellge, G.; Mandić, A. D.; Trautwein, C.; Donath, S. ARC is a novel therapeutic approach against acetaminophen-induced hepatocellular necrosis. *J. Hepatol.* **2013**, *58*, 297–305.
- (34) McKimpson, W. M.; Weinberger, J.; Czerski, L.; Zheng, M.; Crow, M. T.; Pessin, J. E.; Chua, S. C.; Kitsis, R. N. The apoptosis inhibitor ARC alleviates the ER stress response to promote β -cell survival. *Diabetes* **2013**, *62*, 183–193.
- (35) Razorenova, O. V.; Castellini, L.; Colavitti, R.; Edgington, L. E.; Nicolau, M.; Huang, X.; Bedogni, B.; Mills, E. M.; Bogoy, M.; Giaccia, A. J. The apoptosis repressor with a CARD domain (ARC) gene is a direct hypoxia-inducible factor 1 target gene and promotes survival and proliferation of VHL-deficient renal cancer cells. *Mol. Cell. Biol.* **2014**, *34*, 739–751.
- (36) Wang, J. X.; Zhang, X. J.; Feng, C.; Sun, T.; Wang, K.; Wang, Y.; Zhou, L. Y.; Li, P. F. MicroRNA-532-3p regulates mitochondrial fission through targeting apoptosis repressor with caspase recruitment domain in doxorubicin cardiotoxicity. *Cell Death Dis.* **2015**, *6*, e1677.
- (37) Jang, T. H.; Kim, S. H.; Jeong, J. H.; Kim, S.; Kim, Y. G.; Park, H. H. Crystal structure of caspase recruiting domain (CARD) of apoptosis repressor with CARD (ARC) and its implication in inhibition of apoptosis. *Sci. Rep.* **2015**, *5*, 9847.
- (38) Espinosa, J. F.; Gellman, S. H. A designed beta-hairpin containing a natural hydrophobic cluster. *Angew. Chem., Int. Ed.* **2000**, *39*, 2330–2333.