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Arylamide Derivatives as Peptidomimetic Inhibitors of Calmodulin

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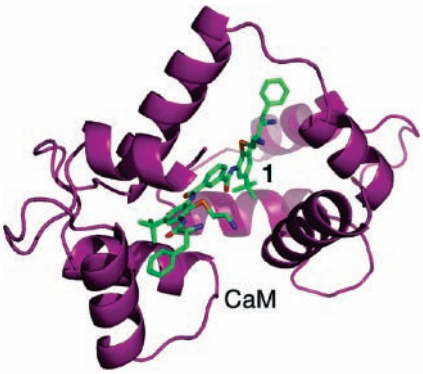
ABSTRACT



Many peptides bind to calmodulin (CaM) in a helical conformation. Here we describe a group of synthetic inhibitors of CaM based on an arylamide scaffold that is intended to mimic smMLCK, a CaM-binding helical peptide. Compound 1 showed a K_i value of 7.10 ± 1.48 nM in a fluorescence polarization assay that monitors the strong association of CaM and its peptide ligand mastoparan X. (^{1}H,^{15}N)-HSQC NMR spectroscopy experiments suggested that 1 binds to CaM in an analogous fashion to that of smMLCK.

Calmodulin (CaM) is a calcium-modulated protein that is abundant in the cytoplasm of all higher cells and has been highly conserved through evolution.[1] CaM is a small dumbbell-shaped protein composed of two globular domains connected by a flexible linker.[2] It mediates the intracellular Ca^{2+} level to the degree of activation of a large number of regulatory proteins, including kinases, phosphatases, and ion channels. Thus it is of much current interest to develop highly potent and specific inhibitors of CaM as it plays important roles in many critical biological processes, such as inflammation, metabolism, apoptosis, muscle contraction, intracellular movement, and short-term and long-term memory.[3] Many small molecules have been previously reported that bind to CaM with high affinity;[4] however, a substantial number of them tend to do so with low specificity and uncertain stoichiometry.[5]

Previous work has shown that CaM binds many targets in helical conformations, such as an α-helical domain of smooth muscle myosin light-chain kinase (smMLCK, K_d = 40.4 ± 13.7 nM),[7] although some peptides, such as the

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(1) Crivici, A.; Ikura, M. *Annu. Rev. Biochem. Biomol. Struct.* **1995**, *24*, 85.
(2) Liddington, R. C. *Nature* **2002**, *415*, 373.
(3) Taules, M.; Rius, E.; Talaya, D.; Lopez-Girona, A.; Bachs, O.; Agell, N. *J. Biol. Chem.* **1998**, *273*, 33279.
(4) For examples, see: (a) Orner, B. P.; Ernst, J. T.; Hamilton, A. D. *J. Am. Chem. Soc.* **2001**, *123*, 5382. (b) Barnes-Seeman, D.; Park, S. B.; Koehler, A. N.; Schreiber, S. L. *Angew. Chem., Int. Ed.* **2003**, *42*, 2376. (c) Yamniuk, A. P.; Vogel, H. J. *Mol. Biotechnol.* **2004**, *27*, 33. (d) Sharma, B.; Deo, S. K.; Bachas, L. G.; Daunert, S. *Bioconjugate Chem.* **2005**, *16*, 1257.
(5) (a) Vandonselaar, M.; Hickie, R. A.; Quail, W.; Delbaere, L. T. J. *Nat. Struct. Biol.* **1994**, *1*, 795. (b) Harmat, V.; Bocskei, Z. S.; Náray-Szabó, G.; Bata, I.; Csutor, A. S.; Hermecz, I.; Arányi, P.; Szabó, B.; Liliom, K.; Vértessy, B. G.; Ovádi, J. *J. Mol. Biol.* **2000**, *297*, 747.

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MARKS peptide, are bound in nonhelical conformations.[8] Mutagenesis studies have established a key role for three residues within smMLCK (Trp800, Thr803, and Val807), at the *i*, *i*+3, and *i*+7 positions, respectively, in binding to the C-terminal domain of CaM in a complex that also involves the collapsed N-terminal region.[9]

We therefore designed a series of CaM inhibitors based on a thioether-substituted arylamide scaffold, which previously has been shown to be a good template for mimicking membrane-binding helical peptides.[10] The thioether substituents in this scaffold help rigidify the conformation through the formation of intramolecular hydrogen bonds, while also providing a site to introduce groups required for molecular recognition. Additional critical substituents can be added to both the amino groups as well as the isophthalic acid ring.[11] Molecular modeling indicated that the *tert*-butyl groups in **1** would match well with hydrophobic side chains in smMLCK, while the two aromatic side chains from the Phe residues would be able to access a deep pocket in each of calmodulin's globular domains (Figure 1).

Arylamide derivatives were prepared using a previously reported modular synthesis (Supporting Information).[12] To prevent proteolytic degradation, D-amino acids with aromatic side chains were used to append to either end of the arylamide backbone.[13] Compound **4** with L-2-naphthylalanine was prepared to compare with **3** to demonstrate the effects of the introduced chiral centers within the amino acid residues. The monocoupled product **5** was obtained as a byproduct in the preparation of **2** due to the incomplete coupling with the pyridylalanine.

A fluorescence polarization (FP) assay has been developed to evaluate the inhibitory effects of these arylamide derivatives in disrupting the CaM−ligand interaction. We have selected mastoparan X (MaX) as the fluorescence probe of the FP assay. MaX (INWKGIAAMAKKLLX) is a tetradecapeptide from the vespid wasp having exceptional affinity for CaM (K_d = 0.3 nM).[10] By monitoring the dissociation of MaX and CaM induced by the arylamide derivatives, the inhibitory potency of these compounds can be determined.

We monitored the intrinsic fluorescence derived from the Trp residue within MaX. The wavelength scanning experiments indicate that the optimal excitation and emission wavelengths are at 292 and 341 nm, respectively. The affinity and 1:1 stoichiometry between CaM and mastoparan X was

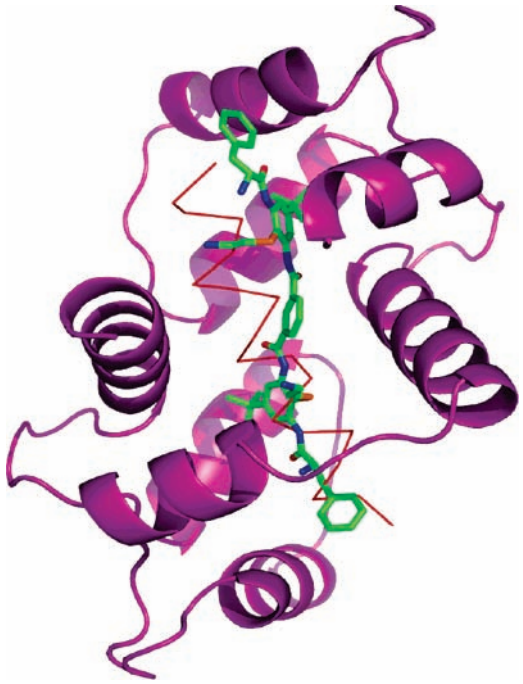


Figure 1. Arylamide **1** designed as a peptidomimetic of the CaM-binding smMLCK helix. Overlay of arylamide **1** (stick) and smMLCK (red ribbon) complexed with CaM (purple cartoon).[6]

confirmed in an experiment in which CaM was titrated into a solution of MaX (Supporting Information Figure S1). Upon titration of CaM into the MaX solution, the maximum emission wavelength of the intrinsic fluorescence shifts from 341 to 327 nm, suggesting that the peptide binding site is either hydrophobic and/or rigid within the protein's interior.[14] With the addition of the arylamide inhibitor, the maximum fluorescence emission shifts back to the original wavelength as the bound peptide is released.

The potency of the arylamide inhibitors was determined in a competition assay in which the 1:1 CaM/MaX complex (0.5 μM) was titrated with increasing concentrations of inhibitors. The dissociation of the CaM/MaX complex was monitored by the increase in polarization as well as the shift in emission maximum. Quantitative analysis of the data showed compound **1**, which has two D-Phe residues, strongly inhibited with an apparent K_i of 7.10 ± 1.48 nM. The naphthylalanine derivatives (**3**, **4**) are less potent than **1** and the D-pyridylalanine derivative **2**, suggesting that single six-membered aromatic rings provide better spatial complementarity than naphthylene groups. The D-2-naphthylalanine analogue **3** gave a K_i of 83.4 ± 6.2 nM, while the L-naphthyl enantiomer **4** was at least 1.4-fold less potent of an inhibitor. The limited solubility of this compound precluded more detailed studies. This result also suggested that the CaM has some stereochemical selectivity in the recognition of these ligands. The control compound **5**, which only displayed half

(6) Molecular modeling studies were conducted using MacPyMOL v0.98 (http://www.pymol.org).

(7) Edwards, R. A.; Walsh, M. P.; Sutherland, C.; Vogel, H. J. *Biochem. J.* **1998**, *331*, 149.

(8) (a) O'Neil, K. T.; DeGrado, W. F. *TIBS* **1990**, *15*, 59. (b) Rhoads, A. R.; Friedberg, F. *FASEB J.* **1997**, *11*, 331.

(9) Meador, W. E.; Means, A. R.; Quiocho, F. A. *Science* **1992**, *257*, 1251.

(10) Tew, G. N.; Liu, D.; Chen, B.; Doerksen, R. J.; Kaplan, J.; Carroll, P. J.; Klein, M. L.; DeGrado, W. F. *Proc. Natl. Aca. Sci. U.S.A.* **2002**, *99*, 5110.

(11) Choi, S.; Clements, D. J.; Pophristic, V.; Ivanov, I.; Vemparala, S.; Bennett, J. S.; Klein, M. L.; Winkler, J. D.; DeGrado, W. F. *Angew. Chem., Int. Ed.* **2005**, *44*, 6685.

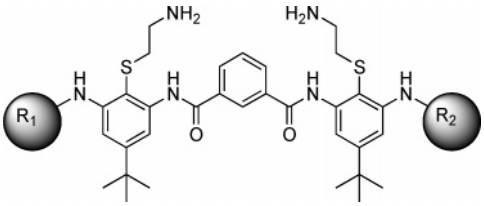
(12) Liu, D.; Choi, S.; Chen, B.; Doerksen, R. J.; Clements, D. J.; Winkler, J. D.; Klein, M. L.; DeGrado, W. F. *Angew. Chem., Int. Ed.* **2004**, *43*, 1158.

(13) Tryptophan was not selected because its emission wavelength overlaps with that of the fluorescence probe peptide.

(14) (a) Malencik, D. A.; Anderson, S. R. *Biochemistry* **1984**, *23*, 2420. (b) Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*, 2nd ed.; Kluwer Academic: New York, 1999; Chapter 1, p 17.

of the functionality of **2**, did not show significant inhibitory effects at concentrations as high as 1 mM, confirming the necessity of the intact arylamide scaffold. We have conducted

Table 1. Results of the Fluorescence Polarization Assay



entry	R_1	R_2	K_i (nM)
1	D-Phe	D-Phe	7.10 ± 1.48
2	D-3-PyA[a]	D-3-PyA	25.7 ± 3.42
3	D-2-Nal[b]	D-2-Nal	83.4 ± 6.2
4	L-2-Nal	L-2-Nal	>120
5	D-3-PyA	H	>1200

[a] PyA is pyridylalanine. [b] Nal is naphthylalanine.

2D-(^{1}H,^{15}N)-HSQC experiments for arylamide derivatives **1** and **2**, which have confirmed that these compounds bind to CaM in a similar way as the CaM-binding peptide smMLCK does. In Figure 2, the spectrum of free CaM is shown in red, CaM in complex with compound **1** is in purple, and CaM in complex with smMLCK is in green. These results show that titrations with **1** and smMLCK affect many of the same residues as indicated by changes in the chemical shift between the free and complexed forms of CaM. For the CaM/**1** complex, 9 residues had significant changes in amide proton chemical shift and 15 residues had significant changes in amide nitrogen chemical shift. Of the sites with perturbed nitrogen chemical shifts, all but two are also significantly perturbed in the CaM/smMLCK complex. This is in marked contrast to the complexes of CaM with the MARCKS family of peptides where available data indicate only one of these sites is perturbed.[15] The fact that many residues were affected suggests that the protein undergoes global conformational changes during the ligand/receptor association, which is consistent with the maximum fluorescence emission changes that occur during MaX binding to CaM. Comparison of the chemical shift changes induced by **1**, smMLCK, and MARCKS confirmed that **1** binds CaM in a fashion similar

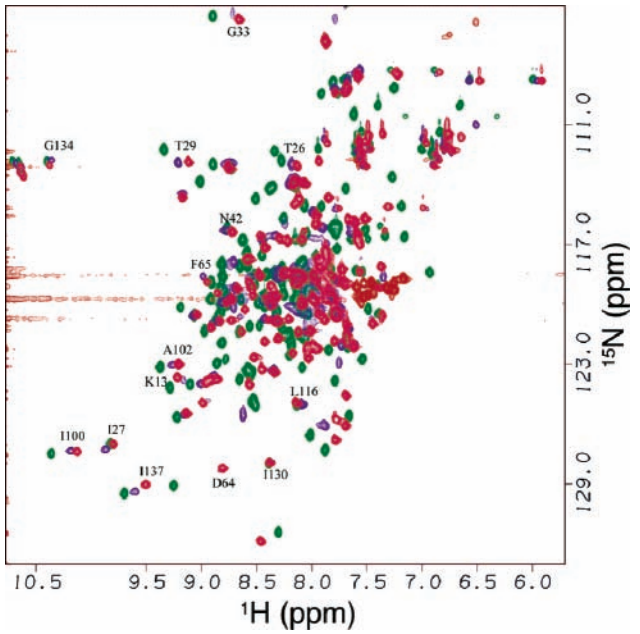


Figure 2. HSQC NMR spectra of the CaM protein (red), in the presence of 2.0 equiv of **1** (purple) and of 1.2 equiv of smMLCK (green).

to the helical CaM ligands (Supporting Information), indicating arylamide derivatives are good peptidomimetics of the α-helical conformation.

In conclusion, we have developed a series of small molecule inhibitors of protein−protein interaction.[16] A group of arylamide derivatives based on structure-based design that disrupt the CaM−ligand association has been identified. These arylamide derivatives have shown inhibitory effects in the lower nanomolar level, and the binding mode of these inhibitors was confirmed using HSQC NMR spectroscopy.

Acknowledgment. We thank the NIH for support of this work. We also thank Dr. David J. Rys for his generous help in the synthesis of the arylamide intermediates.

Supporting Information Available: Experimental details for the FP binding assay, protein preparation, NMR parameters and results. This material is available free of charge via the Internet at http://pubs.acs.org.

OL052478J

(15) Porumb, T.; Crivici, A.; Blackshear, P. J.; Ikura, M. *Biophys. J.* **1997**, *25*, 239.

(16) Yin, H.; Hamilton, A. D. *Angew. Chem., Int. Ed.* **2005**, *44*, 4130.