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Conformationally Locked Calixarene-Based Histone Deacetylase Inhibitors

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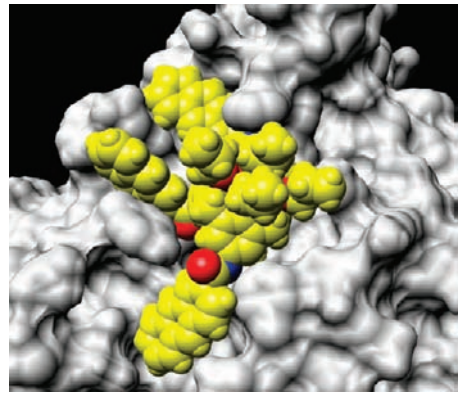
ABSTRACT



Alkyl- and arylamidocalix[4]arene derivatives 1−11 have been designed and theoretically evaluated by docking studies as potential histone deacetylase inhibitors (HDACi). On the basis of the trimodal distribution of the calculated inhibition constants (K_i), five alkyl- or arylamido derivatives (3, 7, 8, 9, and 11) were synthesized and tested. A qualitative accordance between the experimental results and the theoretical predictions was obtained, confirming that appropriately substituted arylamidocalix[4]arenes are active HDACi.

In the past decade, several examples of calixarene derivatives able to interact with molecules of biological interest have been reported.[1] In particular, Hamilton has designed calixarene derivatives able to bind to protein surfaces and to block biologically important protein−protein interactions.[2] Interestingly, the treatment of nude mice bearing human tumors with peptidocalix[4]arene derivatives, able to selectively bind to platelet-derived growth factor (PDGF), resulted in a significant inhibition of tumor growth and angiogenesis.[2c] In addition, it was demonstrated that calixarene-based therapeutic agents do not show any toxic effect in mice tests.[2c,3]

Our group has demonstrated a surface recognition of tissue and microbial transglutaminases by peptidocalix[4]arene diversomers.[4] Larger calix[8]arene derivatives have shown

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(3) Analogously, Liu and co-workers have recently shown that *p*-sulfonatocalix[*n*]arenes do not show toxic effects in mice poisoned with viologen derivatives. In addition, the mortality rate of viologen poisoned mice was significantly decreased: Wang, K.; Guo, D. S.; Zhang, H. Q.; Li, D.; Zheng, X. L.; Liu, Y. *J. Med. Chem.* **2009**, *52*, 6402.

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competitive inhibition of recombinant human tryptase.[5] Water-soluble *p*-sulfonatocalixarenes have shown interesting biological activities, including antiviral, antibacterial, and antithrombotic activity.[6] Recently, Ungaro and co-workers have reviewed the properties of calixarene-based multivalent ligands in lectin binding and inhibition, DNA condensation, and cell transfection.[7]

On this basis, we decided to investigate the use of a calix[4]arene scaffold to construct novel inhibitors of histone deacetylase enzymes (HDACs), and we wish to report here the result of this study.

HDACs are a family of metalloenzymes involved in the remodelling of chromatin, with a key role in the epigenetic regulation of gene expression[8] exerted by removing acetyl groups from lysine or arginine residues[9] in the tails of histones. Since this epigenetic event is associated with carcinogenesis and tumor progression, HDAC inhibitors (HDACi) have been considered promising anticancer agents.[10]

HDACi are generally divided into four groups:[11] short-chain fatty acids, hydroxamic acids, benzamides, and cyclic tetrapeptides. In this field, *Nature* provides a number of related cyclic scaffolds with HDAC inhibitory activity.[12] Even if a large number of structurally diverse HDACi are known, only the pan-HDAC inhibitor Vorinostat has been currently approved by FDA for clinical use in cutaneous T-cell lymphoma.[13]

Notwithstanding the large variety of chemical structures, it is now well established[12] that an efficient HDAC inhibitor should contain three structural features: (I) a hydrophobic region (cap group) involved in the molecular recognition process; (II) a Zn^{II} chelating element (metal binder); and (III) a five to seven atoms spacer (linker) between the cap group and the metal binder. In addition, Ghadiri has recently shown that an appropriate increased structural rigidity enhances the inhibitory activity.[14]

On the basis of these structural considerations,[15] we envisioned that preorganized calix[4]arenes[16] locked in the *cone* conformation, by propoxy groups at the *endo* rim, and endowed with the appropriate functional groups at the *exo* rim to bind to the receptor surface[17] (Figure 1, top) should be ideal candidates as potential HDAC inhibitors.

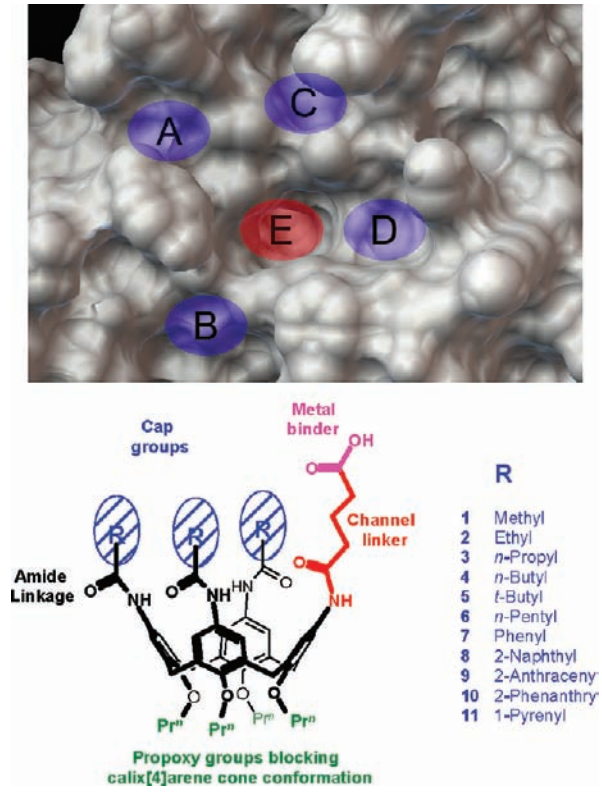

Figure 1. (Top) A−D: Hydrophobic pockets on the HDLP (histone deacetylase like protein) surface. E: Zn^{II} binding channel. (Bottom) Structural features of calix[4]arene derivatives **1**−**11** candidate for HDAC inhibition.

Concerning the functional groups, first, a carbon aliphatic chain entering the binding channel E (channel linker, Figure 1, top) and bearing a metal binder for the Zn^{II} coordination is required (Figure 1, bottom). Second, hydrophobic arms (cap groups, Figure 1, bottom) able to fit the four external hydrophobic pockets A−D (Figure 1, top) on the enzyme surface should be necessary.

To direct the synthesis toward derivatives with higher activity, we performed a molecular docking study of a significant set of designed calix[4]arenes variously substituted at the *exo* rim with aliphatic or aromatic groups of different size and hydrophobicity (Figure 1). In accordance with the current synthetic possibilities, we selected the amide linkage[18] to attach the above moieties at the upper rim of the calixarene scaffold.

Molecular docking studies were articulated in the following steps: (I) choice of the metal binder group and length of the linker chain, (II) choice of the cap groups by gradually

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increasing the length of an amide aliphatic chain (from one to five carbon atom), and (III) choice of aromatic cap groups by gradually increasing the size of the aromatic system (from one to four condensate rings). To rationalize and to identify the structural features of each calixarene derivative, we performed molecular docking studies, by using the Autodock 3.0.5 program,[19] on the HDLP (histone deacetylase like protein, PDB code 1C3R) binding site (Figure 1, top).[20] For the linker chain and metal binder, we referred to Class I selective HDAC inhibitor Azumamide E,[21] and we used a six-membered chain including a terminal carboxy functionality. This latter is usually considered dissociated at physiological pH, and consequently, we used its deprotonated form in the calculations. Subsequently, we systematically conjugated the amide linker at the upper rim with different alkylic and aromatic groups to optimize the size and the chemicophysical properties of the cap group. In Figure 2 we report

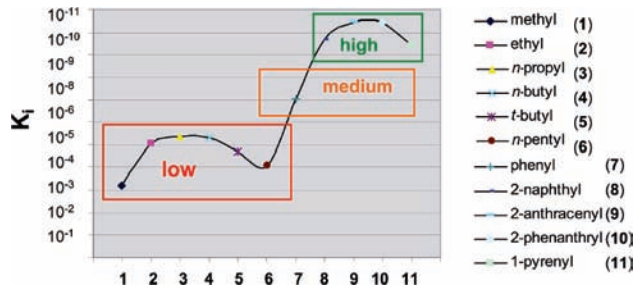

Figure 2. Variation of the final calculated inhibition constant (K_i) as a function of different alkylic and aromatic groups connected at the amide linker at the upper rim of calix[4]arenes **1**−**11**.

the calculated affinity, expressed by a theoretical inhibition constant (K_i) of molecules **1**−**11** (Figure 1) for the HDLP target.[22] As it is possible to observe, the K_i values are strictly dependent on the size of the arms. In particular, we can divide the curve in three different zones: low, medium, and high theoretical activity.

Interestingly, the low zone corresponds to amidocalix[4]-arenes **1**−**6** bearing alkylic arms, whereas the medium and high zone of Figure 2 correspond to the series of amidocalixarenes **7**−**11** bearing aromatic arms of increasing size (see Figures S1−S3 in the Supporting Information for the putative three-dimensional models of their HDLP complexes).

For the sake of simplicity, we describe here the 3D model of the most promising candidate **9** (Figure 3) to pin down the main features of new potential calixarene-based HDACi. The linker chain of **9** fits into the 11 Å binding channel, and the carboxylate moiety binds to the Zn^{II} ion, at the bottom of the channel, in a bidentate fashion establishing hydrogen

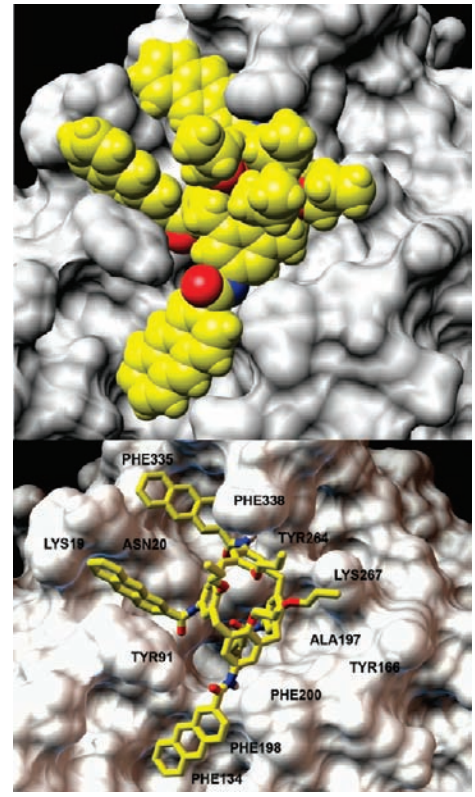

Figure 3. 3D model of the putative binding mode of amidocalix[4]arene **9** and the HDLP binding site. The protein is represented by its molecular surface (gray), while **9** is depicted as a CPK (top) or stick model (bottom) (colored by atom type: C, yellow, polar H, white; N, dark blue; O, red).

bonds with $H^{\varepsilon 2}$ of His132; moreover, an amide function forms an additional hydrogen bond with $N^{\delta 1}$ of HIS170. The aromatic arms of **9** occupy the pockets A, B, and C establishing van der Waals interactions with the enzyme counterpart, a π-stacking interaction with TYR91 (pocket A) and TYR264 (pocket C), and a cation−π interaction with LYS19 (pocket A). Our results show that the influence of the groups at the upper rim is mainly related to hydrophobic or aromatic stacking interactions, which seem to be among the main driving forces of the target-ligand complexes.

To verify the above in silico results, we decided to check the key points of the predicted curve represented in Figure 2: the most active candidate of the low zone (**3**), the intermediate (**7**), and three derivatives of the high zone, namely, **8**, **9**, and **11**.

The five derivatives **3**, **7**, **8**, **9**, and **11** were synthesized exploiting a common reaction sequence (Scheme 1)[22] in which an easily obtained starting tetraaminocalix[4]arene **12**,[23] already locked in the cone conformation by the four propoxy groups at the endo rim, is selectively mono-Boc-protected, according to the procedure reported by Böhmer, to give derivative **13**.[24]

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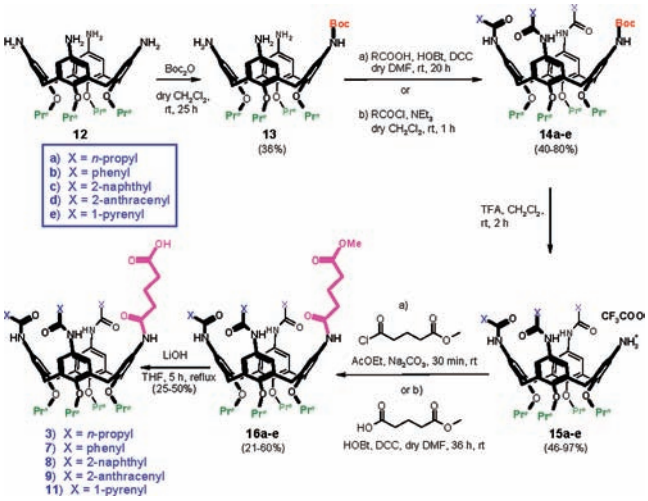
Scheme 1. [22]



The three free amino groups of this latter can then be aroylated using the corresponding aroyl chlorides (benzoyl, 2-naphthoyl, for derivatives **7** and **8**, respectively) in the presence of Et_3N to afford Boc-protected triarylamidocalix[4]-arenes **14b**,**c** in good yields. Derivatives **14a**,**d**,**e** were instead obtained by coupling **13** with butyric, 2-anthracenic, and 1-pyrenic acid, respectively, in the presence of DCC and HOBt in dry DMF as solvent. The subsequent deprotection with TFA in CH_2Cl_2 gave monoamino derivatives **15a**−**e** in good yields. Derivatives **15b**,**c**,**e** were coupled in the presence of Na_2CO_3 with the 1,5-pentandioic acid chloride monoprotected as methyl ester to give **16b**,**c**,**e**, while derivatives **16a**,**d** were readily obtained by coupling **15a**,**d** with monomethylglutaric acid, in the presence of DCC and HOBt in dry DMF as solvent. The LiOH-mediated hydrolysis of the estereal function straightly led to the desired derivatives **3**, **7**, **8**, **9**, and **11**.[22]

In vitro evaluation of inhibition of HDAC activity in HeLa nuclear extracts was performed by a fluorescence-based assay.[22] The IC_{50} values of compounds **3**, **7**, **8**, **9**, **11**, and Trichostatin A (TSA), a well-known HDAC inhibitor, are reported in Table 1.

As predicted by the docking studies, alkyl derivative **3** was the less active compound ($IC_{50} > 10\ \mu M$) followed by phenyl derivative **7** ($IC_{50} = 5.10\ \mu M$). On the other hand, **8**, **9**, and **11**, bearing larger aromatic rings, displayed higher inhibitory activities ($IC_{50} = 0.14-0.86\ \mu M$), although with less pronounced differences with respect to the predicted ones. Probably the calculated differences fall within the accuracy limit of the docking method. In any case, these

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Table 1. In Vitro HDAC Inhibitory Activity ($IC_{50} \pm$ sd)

compound	IC_{50} (μM)[a]
3	> 10
7	5.10 (±1.00)
8	0.14 (±0.02)
9	0.14 (±0.02)
11	0.86 (±0.10)
TSA	0.02 (±0.009)

[a] Values are means of three independent experiments. Standard deviation values were < 20% and are reported in parentheses.

results confirm that topology, size, and hydrophobicity of the aromatic arms are the most important determinants for biological activity of this novel class of calix[4]arene inhibitors.

In conclusion, we have applied a classic in silico screening of a new class of potential HDAC inhibitors to give good predictions of their inhibitory activity before proceeding to their synthesis. In this way, it was possible to design a new class of amidocalix[4]arenes permanently locked in a cone conformation with convergently predisposed interacting moieties. The in silico evaluation of their binding ability toward the HDAC active site allowed us to direct the synthesis only to the most promising candidates, thus avoiding a useless waste of resources.

The subsequent synthesis and enzyme inhibition evaluation fully confirmed the theoretical prediction that arylamidocalix[4]-arenes bearing large aromatic arms constitute moderately active HDACi. Considering that the calixarene frameworks had shown no hint of toxicity in several in vivo biological tests,[2c,3] this work suggests an additional application of a scaffold already used in the fields of biomolecular recognition.

Future work will be directed toward the in silico screening of nonsymmetrically substituted arylamidocalix[4]arenes that could give an even better fitting on the different enzyme hydrophobic A−D pockets. The influence of the "cone-blocking" groups at the lower rim will also be evaluated as well as the possibility to introduce groups able to increase their water solubility.

Acknowledgment. Dr. Patrizia Iannece (Dipartimento di Chimica, Università di Salerno) for ESI-MS measurements.

Supporting Information Available: Synthetic details, 1D and 2D NMR, ESI(+) MS, and UV−vis spectra. Details on molecular docking and Cartesian coordinates of compounds **1**−**11** bound to HDLP. Experimental details on in vitro evaluation of HDAC inhibition activity. This material is available free of charge via the Internet at http://pubs.acs.org.

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