Year of publication 2020

Thomas Barbot, Veronica Beswick, Cédric Montigny, Éric Quiniou, Nadège Jamin and Liliane Mouawad (2020 Nov 6)

Deciphering the mechanism of inhibition of SERCA1a by sarcolipin using molecular simulations

Frontiers in Molecular Biosciences : Accepted article : DOI : 10.3389/fmolb.2020.606254

Summary

SERCA1a is an ATPase calcium pump that transports Ca2+ from the cytoplasm to the sarco/endoplasmic reticulum lumen. Sarcolipin (SLN), a transmembrane peptide, regulates the activity of SERCA1a by decreasing its Ca2+ transport rate, but its mechanism of action is still not well understood. To decipher this mechanism, we have performed normal mode analysis in the all-atom model, with the SERCA1a-SLN complex, or the isolated SERCA1a, embedded in an explicit membrane. The comparison of the results allowed us to provide an explanation at the atomic level for the action of SLN that is in good agreement with experimental observations. In our analyses, the presence of SLN locally perturbs the TM6 transmembrane helix and as a consequence modifies the position of D800, one of the key metal-chelating residues. Additionally, it reduces the flexibility of the gating residues, V304 and E309 in TM4, at the entrance of the Ca2+ binding sites, which would decrease the affinity for Ca2+. Unexpectedly, SLN has also an effect on the ATP binding site more than 35 Å away, due to the straightening of TM5, a long helix considered as the spine of the protein. The straightening of TM5 modifies the structure of the P-N linker that sits above it, and which comprises the 351DKTG354 conserved motif, resulting in an increase of the distance between ATP and the phosphorylation site. As a consequence, the turn-over rate could be affected. All this gives SERCA1a the propensity to go toward a Ca2+ low-affinity E2-like state in the presence of SLN and toward a Ca2+ high-affinity E1-like state in the absence of SLN. In addition to a general mechanism of inhibition of SERCA1a regulatory peptides, this study also provides an insight into the conformational transition between the E2 and E1 states.

Küssau T., Van Wyk N., Johansen M.D., Alsarraf H.M.A.B., Neyret A., Hamela C., Sørensen K.K., Thygesen M.B., Beauvineau C., Kremer L., Blaise M. (2020 Nov 4)

Functional Characterization of the N-Acetylmuramyl-l-Alanine Amidase, Ami1, from Mycobacterium abscessus

Cells : 9 : 2410 : DOI : 10.3390/cells9112410

Summary

Peptidoglycan (PG) is made of a polymer of disaccharides organized as a three-dimensional mesh-like network connected together by peptidic cross-links. PG is a dynamic structure that is essential for resistance to environmental stressors. Remodeling of PG occurs throughout the bacterial life cycle, particularly during bacterial division and separation into daughter cells. Numerous autolysins with various substrate specificities participate in PG remodeling. Expression of these enzymes must be tightly regulated, as an excess of hydrolytic activity

can be detrimental for the bacteria. In non-tuberculous mycobacteria such as Mycobacterium abscessus, the function of PG-modifying enzymes has been poorly investigated. In this study, we characterized the function of the PG amidase, Ami1 from M. abscessus. An ami1 deletion mutant was generated and the phenotypes of the mutant were evaluated with respect to susceptibility to antibiotics and virulence in human macrophages and zebrafish. The capacity of purified Ami1 to hydrolyze muramyl-dipeptide was demonstrated in vitro. In addition, the screening of a 9200 compounds library led to the selection of three compounds inhibiting Ami1 in vitro. We also report the structural characterization of Ami1 which, combined with in silico docking studies, allows us to propose a mode of action for these inhibitors.

Breton-Patient C., Naud-Martin D., Mahuteau-Betzer F., Piguel S. (2020 Oct 14)

Three-component C-H bond sulfonylation of imidazoheterocycles via visible-light organophotoredox catalysis.

European Journal of Organic Chemistry : Accepted Article : DOI : 10.1002/ejoc.202001219

Summary

The first entirely visible-light photoredox catalyzed sulfonylation of imidazoheterocycles has been developed. This transformation demonstrates an efficient C-H functionalization for the straightforward synthesis of novel C-3 sulfonylated imidazoheterocycles from various imidazopyridines and diaryliodonium salts with different electronic and steric properties and easy handled DABCO- bis (sulfur dioxide). The reaction proceeds in moderate to good yields under mild conditions at room temperature using the inexpensive organophotocatalyst EosinY.Na 2 and shows a high functional group tolerance (37 examples).

Marchand A., Beauvineau C., Teulade-Fichou M.P., Zenobi R. (2020 Oct 14)

Competition of ligands and the 18-mer binding domain of the RHAU helicase for G-quadruplexes – orthosteric or allosteric binding mechanism?

Chemistry – A European Journal : Accepted article : DOI : 10.1002/chem.202004040

Summary

Stabilizing particular DNA and RNA structures called G-quadruplexes (G4s) using specific ligands (L) is a strategy proposed to fight cancer. However, while G4:L interactions are often investigated, whether or not ligands are able to disrupt interactions between G4s and proteins (P) remains poorly studied. Here, using native mass spectrometry, we investigated ternary G4:L:P complexes formed by G4s, some of the highest affinity ligands, and the binding domain of the RHAU helicase. First, our results suggest that RHAU binds not only preferentially to parallel G4s but to free external G-quartets. We also found that, depending on the G4, ligands could prevent the binding of the peptide, either by direct competition for the binding sites (orthosteric inhibition) or by inducing conformational changes (allosteric inhibition). Notably, the ligand Cu-ttpy induced a conformational change that increased the binding of the peptide. This study illustrates that it is important to not only characterize drug-target interactions but also how the binding to other partners is affected.

Sandra Cunha Silveira, Géraldine Buhagiar-Labarchède, Rosine Onclercq-Delic, Simon Gemble, Elias Bou Samra, Hamza Mameri, Patricia Duchambon, Christelle Machon, Jérôme Guitton & Mounira Amor-Guéret (2020 Aug 17)

A decrease in NAMPT activity impairs basal PARP-1 activity in cytidine deaminase deficient-cells, independently of NAD^+

Scientific Reports : 10 : 13907 : DOI : 10.1038/s41598-020-70874-6

Summary

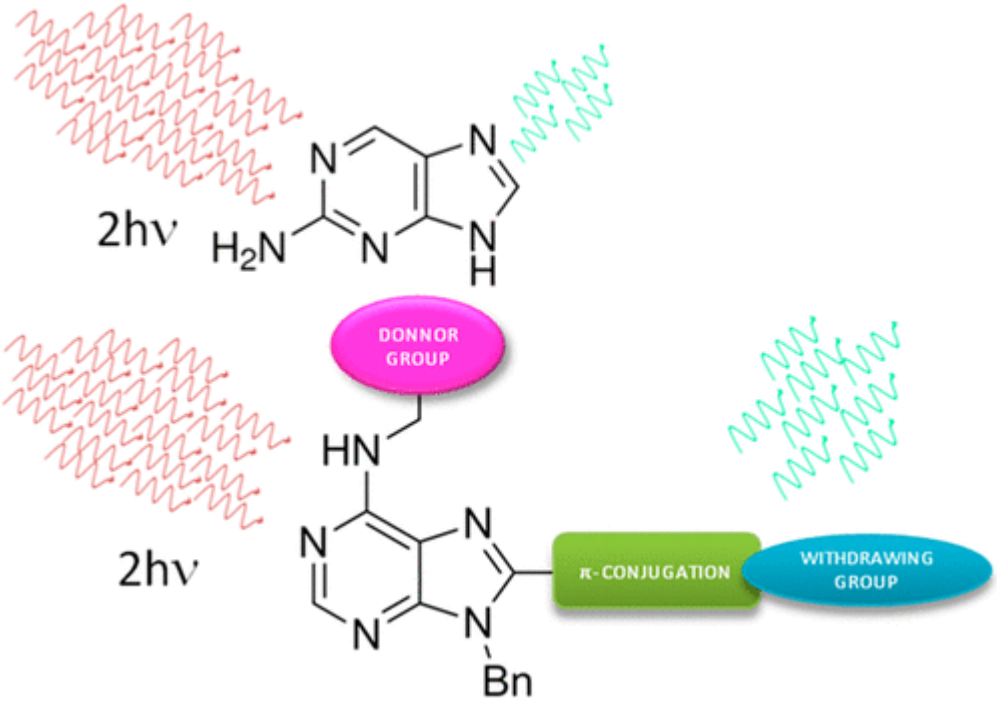
Cytidine deaminase (CDA) deficiency causes pyrimidine pool disequilibrium. We previously reported that the excess cellular dC and dCTP resulting from CDA deficiency jeopardizes genome stability, decreasing basal poly(ADP-ribose) polymerase 1 (PARP-1) activity and increasing ultrafine anaphase bridge (UFB) formation. Here, we investigated the mechanism underlying the decrease in PARP-1 activity in CDA-deficient cells. PARP-1 activity is dependent on intracellular NAD^+ concentration. We therefore hypothesized that defects of the NAD^+ salvage pathway might result in decreases in PARP-1 activity. We found that the inhibition or depletion of nicotinamide phosphoribosyltransferase (NAMPT), the rate-limiting enzyme in the NAD^+ salvage biosynthesis pathway, mimicked CDA deficiency, resulting in a decrease in basal PARP-1 activity, regardless of NAD^+ levels. Furthermore, the expression of exogenous wild-type NAMPT fully restored basal PARP-1 activity and prevented the increase in UFB frequency in CDA-deficient cells. No such effect was observed with the catalytic mutant. Our findings demonstrate that (1) the inhibition of NAMPT activity in CDA-proficient cells lowers basal PARP-1 activity, and (2) the expression of exogenous wild-type NAMPT, but not of the catalytic mutant, fully restores basal PARP-1 activity in CDA-deficient cells; these results strongly suggest that basal PARP-1 activity in CDA-deficient cells decreases due to a reduction of NAMPT activity.

Leandro H. Zucolotto Cocca, Luis M. G. Abegão, Lucas F. Sciuti, Roxane Vabre, Jonathas de Paula Siqueira, Kenji Kamada, Cleber R. Mendonca, Sandrine Piguel, and Leonardo De Boni (2020 Jun 11)

Two-Photon Emissive Dyes Based on Push–Pull Purines Derivatives: Toward the Development of New Photoluminescence Bioprobes

The Journal of Physical Chemistry C : 124 : 12185-12864 : DOI : 10.1021/acs.jpcc.0c01859

Summary



Fluorescent organic molecules have received great attention due to their largest applications, for example, in DNA and RNA spectroscopies studies, development of new photoluminescence bioprobes, and applications in fluorescence spectroscopy. In specific, purine base analog molecules present high fluorescence quantum yields and significant Stokes shift. Furthermore, the addition of push–pull structures at the purine core could increase the photoluminescence properties, making candidates for photoluminescence bioprobes. To consider this, a complete spectroscopic study was performed on nine push–pull purines, distinguished by different push–pull structures. In specific, for this research, the two-photon absorption (2PA) study showed that the compounds present induced two-photon fluorescence at the therapeutic window, desired for fluorescence microscopy. The brightness property was evaluated, indicating that all chromospheres are fluorescent by a 2PA process. Additionally, ultrafast transient absorption was performed to elucidate contribution of the excited states on the 2PA spectra, and quantum chemistry calculations were performed to corroborate the experimental results.

Rahima Chennoufi, Ngoc-Duong Trinh, Françoise Simon, Guillaume Bordeau, Delphine Naud-Martin, Albert Moussaron, Bertrand Cinquin, Houcine Bougherara, Béatrice Rambaud, Patrick Tauc, Céline Frochot, Marie-Paule Teulade-Fichou, Florence Mahuteau-Betzer & Eric Deprez (2020 Apr 23)

Interplay between cellular uptake, intracellular localization and the cell death mechanism in triphenylamine-mediated photoinduced cell death

Scientific Reports : 10 : 6881 : DOI : 10.1038/s41598-020-63991-9

Summary

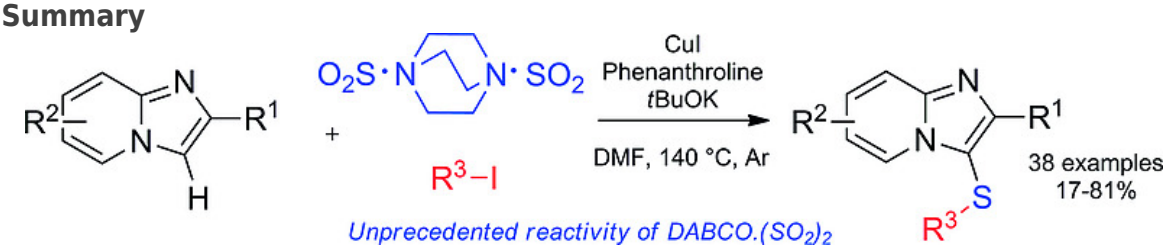
Triphenylamines (TPAs) were previously shown to trigger cell death under prolonged one- or two-photon illumination. Their initial subcellular localization, before prolonged illumination, is exclusively cytoplasmic and they translocate to the nucleus upon photoactivation. However, depending on their structure, they display significant differences in terms of precise initial localization and subsequent photoinduced cell death mechanism. Here, we investigated the structural features of TPAs that influence cell death by studying a series of molecules differing by the number and chemical nature of vinyl branches. All compounds triggered cell death upon one-photon excitation, however to different extents, the nature of the electron acceptor group being determinant for the overall cell death efficiency. Photobleaching susceptibility was also an important parameter for discriminating efficient/inefficient compounds in two-photon experiments. Furthermore, the number of branches, but not their chemical nature, was crucial for determining the cellular uptake mechanism of TPAs and their intracellular fate. The uptake of all TPAs is an active endocytic process but two- and three-branch compounds are taken up via distinct endocytosis pathways, clathrin-dependent or -independent (predominantly caveolae-dependent), respectively. Two-branch TPAs preferentially target mitochondria and photoinduce both apoptosis and a proper necrotic process, whereas three-branch TPAs preferentially target late endosomes and photoinduce apoptosis only.

Julie Le Bescont, Chloé Breton-Patient et Sandrine Piguel (2020 Apr 16)

Unconventional Reactivity with DABCO-Bis(sulfur dioxide): C–H Bond Sulfenylation of Imidazopyridines

European Journal of Organic Chemistry : 2020 : 2101-2109 : DOI : 10.1002/ejoc.202000112

Summary



Exploring the unexpected reactivity of DABCO-bis(sulfur dioxide) on various imidazo[1,2-a]pyridines expanded the toolbox of the sulfenylation reagent. Starting from three simple building blocks, this three-component transformation led to various C-3 sulfenylated substituted imidazo[1,2-a]pyridines in moderate to good yields.

This work highlights the unexpected and unprecedented outcome of the reactivity with DABCO-bis(sulfur dioxide). The use of this reagent led to the exclusive introduction of a sulfur atom on the C-3 position of imidazopyridines instead of a sulfone group. The reaction methodology turned out to be robust, scalable and suitable for various imidazopyridines and aryl iodides both bearing substituents with different electronic and steric properties (38 examples). Beyond the fact that this synthetic method complements the previously reported protocols for sulfenylation reactions, this work is meant to underline the unconventional role

of DABCO-bis(sulfur dioxide).

Year of publication 2019

Abegão L.M.G., Fonseca R.D., Santos F.A., Rodrigues J.J., Kamada K., Mendonça C.R., Piguel S., De Boni L. (2019 Aug 23)

First molecular electronic hyperpolarizability of series of π-conjugated oxazole dyes in solution: an experimental and theoretical study

RSC Adv. : 9 : 26476-26482 : DOI : 10.1039/C9RA05246A

Summary

In this work, we report the experimental and theoretical first molecular electronic hyperpolarizability (βHRS) of eleven π-conjugated oxazoles compounds in toluene medium. The Hyper-Rayleigh Scattering (HRS) technique allowed the determination of the experimental dynamic βHRS values, by exciting the compounds with a picosecond pulse trains from a Q-switched and mode-locked Nd:YAG laser tuned at 1064 nm. Theoretical predictions based on time-dependent density functional theory level using the Gaussian 09 program package were performed with three different functionals (B3LYP, CAM-B3LYP, and M06-2X), to calculate both static and dynamic theoretical βHRS values. Good accordance was found between the experimental and theoretical values, in particular for the CAM-B3LYP and M06-2X functionals.

El Hassen Mokrani, Abderrahmane Bensegueni, Ludovic Chaput, Claire Beauvineau, Hanane Djeghim, Liliane Mouawad (2019 May 1)

Identification of New Potent Acetylcholinesterase Inhibitors Using Virtual Screening and *In Vitro* Approaches.

Molecular informatics : 38 : 1800118 : DOI : 10.1002/minf.201800118

Summary

Acetylcholinesterase (AChE) is currently the most favorable target for the symptomatic treatment and reduction of Alzheimer's disease (AD). In order to identify new potent inhibitors of this enzyme, we describe herein a new structure-based virtual screening (SBVS) using the Institut Curie-CNRS chemical library (ICCL), which contained at the screening date 14307 compounds. The strategy undertaken in this work consisted of the use of several docking programs in SBVS calculations followed by the application of a consensus method (vSDC) and a scrupulous visual analysis. It allowed us to obtain a high degree of success, with a yield of almost 86 %, since 12 hits were identified among only 14 molecules tested *in vitro*. Still more remarkably, 6 of these hits were more active than galantamine, the reference inhibitor. These hits were predicted to have good ADMET properties. The two most promising compounds can serve as leads for AD treatment.

Morgan Pellerano, Delphine Naud-Martin, Florence Mahuteau-Betzer, Marie Morille, May Catherine Morris (2019 Feb 15)

Fluorescent biosensor for detection of the R248Q aggregation-prone mutant of p53.

Chembiochem : a European journal of chemical biology : 20 : 605-613 : DOI : 10.1002/cbic.201800531

Summary

The p53 tumour suppressor and guardian of the genome undergoes missense mutations which lead to functional inactivation in 50% human cancers. These mutations occur mostly in the DNA-binding domain of the protein and several of these induce conformational changes which lead to amyloid-like protein aggregation. Here we describe a fluorescent biosensor that reports on the R248Q mutant of p53 in vitro and in living cells, engineered through conjugation of an environmentally-sensitive probe onto a peptide derived from the primary aggregation segment of p53.This biosensor was characterized both in vitro and by fluorescence microscopy following facilitated delivery into cultured cells. We show that this biosensor preferentially reports on the p53 R248Q mutant in PC9 lung cancer cell line compared to other lung cancer cell lines harbouring either wildtype or no p53.

Pauline Gilson, Morgane Couvet, Laetitia Vanwonterghem, Maxime Henry, Julien Vollaire, Vladimir Baulin, Marco Werner, Anna Orlowska, Véronique Josserand, Florence Mahuteau-Betzer, Laurence Lafanechère, Jean-Luc Coll, Benoit Busser, Amandine Hurbin (2019 Feb 1)

The pyrrolopyrimidine colchicine-binding site agent PP-13 reduces the metastatic dissemination of invasive cancer cells *in vitro* and *in vivo*.

Biochemical pharmacology : 160 : 1-13 : DOI : S0006-2952(18)30503-3

Summary

Standard chemotherapies that interfere with microtubule dynamics are a chemotherapeutic option used for the patients with advanced malignancies that invariably relapse after targeted therapies. However, major efforts are needed to reduce their toxicity, optimize their efficacy, and reduce cancer chemoresistance to these agents. We previously identified a pyrrolo[2,3d]pyrimidine-based microtubule-depolymerizing agent (PP-13) that binds to the colchicine site of β-tubulin and exhibits anticancer properties in solid human cancer cells, including chemoresistant subtypes. Here, we investigated the therapeutic potential of PP-13 in vitro and in vivo. PP-13 induced a mitotic blockade and apoptosis in several cancer cells cultured in two-dimensions or three-dimensions spheroids, in conjunction with reduced cell proliferation. Capillary-like tube formation assays using HUVECs showed that PP-13 displayed antiangiogenic properties. It also inhibited cancer cell motility and invasion, in in vitro wound-healing and transwell migration assays. Low concentration PP-13 (130 nmol.L) treatment significantly reduced the metastatic invasiveness of human cancer cells engrafts on chicken chorioallantoic membrane. In nude mice, 0.5 or 1 mg.kg PP-13 intraperitoneally administered three-times a week reduced the sizes of paclitaxel-refractory orthotopic breast tumors, delayed the progression of metastasis, and decreased the global metastatic load

compared to 0.5 mg.kg paclitaxel or vehicle alone. PP-13 did not show any apparent early adverse effect in vivo. These data suggest that PP-13 is a promising alternative to standard chemotherapy in antimitotic drug-refractory tumors, especially through its impact on metastasis.

Delphine Naud-Martin, Corinne Landras-Guetta, Daniela Verga, Deepanjan Ghosh, Sylvain Achelle, Florence Mahuteau-Betzer, Sophie Bombard, Marie-Paule Teulade-Fichou (2019 Jan 26)

Selectivity of Terpyridine Platinum Anticancer Drugs for G-quadruplex DNA.

Molecules (Basel, Switzerland) : 24 : 404 : DOI : 10.3390/molecules24030404

Summary

Guanine-rich DNA can form four-stranded structures called G-quadruplexes (G4s) that can regulate many biological processes. Metal complexes have shown high affinity and selectivity toward the quadruplex structure. Here, we report the comparison of a panel of platinum (II) complexes for quadruplex DNA selective recognition by exploring the aromatic core around terpyridine derivatives. Their affinity and selectivity towards G4 structures of various topologies have been evaluated by FRET-melting (Fluorescence Resonance Energy Transfert-melting) and Fluorescent Intercalator Displacement (FID) assays, the latter performed by using three different fluorescent probes (Thiazole Orange (TO), TO-PRO-3, and PhenDV). Their ability to bind covalently to the c-myc G4 structure in vitro and their cytotoxicity potential in two ovarian cancerous cell lines were established. Our results show that the aromatic surface of the metallic ligands governs, in vitro, their affinity, their selectivity for the G4 over the duplex structures, and platination efficiency. However, the structural modifications do not allow significant discrimination among the different G4 topologies. Moreover, all compounds were tested on ovarian cancer cell lines and normal cell lines and were all able to overcome cisplatin resistance highlighting their interest as new anticancer drugs.

M Schmidt-Cernohorska, I Zhernov, E Steib, M Le Guennec, R Achek, S Borgers, D Demurtas, L Mouawad, Z Lansky, V Hamel, P Guichard (2019 Jan 19)

Flagellar microtubule doublet assembly *in vitro* reveals a regulatory role of tubulin C-terminal tails.

Science (New York, N.Y.) : 363 : 285-288 : DOI : 10.1126/science.aav2567

Summary

Microtubule doublets (MTDs), consisting of an incomplete B-microtubule at the surface of a complete A-microtubule, provide a structural scaffold mediating intraflagellar transport and ciliary beating. Despite the fundamental role of MTDs, the molecular mechanism governing their formation is unknown. We used a cell-free assay to demonstrate a crucial inhibitory role of the carboxyl-terminal (C-terminal) tail of tubulin in MTD assembly. Removal of the C-terminal tail of an assembled A-microtubule allowed for the nucleation of a B-microtubule on its surface. C-terminal tails of only one A-microtubule protofilament inhibited this side-to-

surface tubulin interaction, which would be overcome in vivo with binding protein partners. The dynamics of B-microtubule nucleation and its distinctive isotropic elongation was elucidated by using live imaging. Thus, inherent interaction properties of tubulin provide a structural basis driving flagellar MTD assembly.

Year of publication 2018

Tom Baladi, Jessy Aziz, Florent Dufour, Valentina Abet, Véronique Stoven, François Radvanyi, Florent Poyer, Ting-Di Wu, Jean-Luc Guerquin-Kern, Isabelle Bernard-Pierrot, Sergio Marco Garrido, Sandrine Piguel (2018 Nov 1)

Design, synthesis, biological evaluation and cellular imaging of imidazo[4,5-b]pyridine derivatives as potent and selective TAM inhibitors.

Bioorganic & medicinal chemistry : 26 : 5510-5530 : DOI : 10.1016/j.bmc.2018.09.031

Summary

The TAM kinase family arises as a new effective and attractive therapeutic target for cancer therapy, autoimmune and viral diseases. A series of 2,6-disubstituted imidazo[4,5-b]pyridines were designed, synthesized and identified as highly potent TAM inhibitors. Despite remarkable structural similarities within the TAM family, compounds 28 and 25 demonstrated high activity and selectivity in vitro against AXL and MER, with IC value of 0.77 nM and 9 nM respectively and a 120- to 900-fold selectivity. We also observed an unexpected nuclear localization for compound 10Bb, thanks to nanoSIMS technology, which could be correlated to the absence of cytotoxicity on three different cancer cell lines being sensitive to TAM inhibition.