Publications de l’équipe
Chimie des biomolécules, des sondes et des inhibiteurs hétérocycliques

Année de publication : 2018

Silvia Serra, Ahmed Alouane, Thomas Le Saux, Steve Huvelle, Raphaël Plasson, Frédéric Schmidt, Ludovic Jullien, Raphaël Labruère (2018 Jun 7)
A chemically encoded timer for dual molecular delivery at tailored ranges and concentrations.
Chemical communications (Cambridge, England) : 6396-6399 : DOI : 10.1039/c8cc03253j

Résumé

Spatiotemporal control of molecular distribution is much in demand in many fields of chemistry. To address this goal, we exploit a low molecular weight branched self-immolative architecture, which acts as a triggerable chemically encoded timer for autonomous sequential release of two chemicals. Using a light-activated model liberating two distinct fluorophores, we generated a tunable spatially contrasted molecular distribution.

Année de publication : 2017

Steve Huvelle, Ahmed Alouane, Thomas Le Saux, Ludovic Jullien, Frédéric Schmidt (2017 Mar 31)
Syntheses and kinetic studies of cyclisation-based self-immolative spacers.
Organic & biomolecular chemistry : 3435-3443 : DOI : 10.1039/c7ob00121e

Résumé

Kinetic analysis of the disassembly of self-immolative spacers based on cyclisation processes was performed. Five compounds were synthesized belonging to two different series, and their kinetic constants were determined. Electron-donating substituents gave a slight acceleration but the main effect was steric, and the Thorpe-Ingold effect was indeed particularly effective. Comparison with the self-immolative spacers based on elimination processes showed that cyclisations gave comparable or lower rate, but the corresponding spacers are more difficult to modulate.

Guillaume Kellermann, Florent Dingli, Vanessa Masson, Daniel Dauzonne, Evelyne Ségal-Bendirdjian, Marie-Paule Teulade-Fichou, Damarys Loew, Sophie Bombard (2017 Mar 1)
Exploring the mechanism of inhibition of human telomerase by cysteine-reactive compounds

Résumé

Telomerase is an almost universal cancer target that consists minimally of a core protein (hTERT) and an RNA (hTR). Some inhibitors of this enzyme are thought to function by the covalent binding to one or several cystein residues; however, this inhibition mechanism has
never been investigated because of the difficulty in producing telomerase. In the present study, we use a recent method to produce recombinant hTERT to analyse the effect of cysteine reactive inhibitors on telomerase. Using mass-spectrometry (MS) and mutagenesis analysis, we identify several targeted residues in separated domains of the hTERT protein and show that cysteine-reactive reagents abolish the interaction with the CR4/5 region of hTR. This article is protected by copyright. All rights reserved.

Année de publication : 2016


Oncoimmunology : e1171434 : DOI : 10.1080/2162402X.2016.1171434

Résumé

Antibody-drug conjugates (ADC), combining the specificity of tumor recognition by monoclonal antibodies (mAb) and the powerful cytotoxicity of anticancer drugs, are currently under growing interest and development. Here, we studied the potential of Chi-Tn, a mAb directed to a glyco-peptidic tumor-associated antigen, to be used as an ADC for cancer treatment. First, we demonstrated that Chi-Tn specifically targeted tumor cells in vivo. Also, using flow cytometry and deconvolution microscopy, we showed that the Chi-Tn mAb is rapidly internalized - condition necessary to ensure the delivery of conjugated cytotoxic drugs in an active form, and targeted to early and recycling endosomes. When conjugated to saporin (SAP) or to auristatin F, the Chi-Tn ADC exhibited effective cytotoxicity to Tn-positive tumor cells in vitro, which correlated with the level of tumoral Tn expression. Furthermore, the Chi-Tn mAb conjugated to auristatin F also exhibited efficient antitumor activity in vivo, validating for the first time the use of an anti-Tn antibody as an effective ADC.


Chemistry (Weinheim an der Bergstrasse, Germany) : DOI : 10.1002/chem.201603003

Résumé

C(sp)-H bond activation of acetylene molecule still remains a challenge for synthetic organic chemist. In practice, it is activated by strong base and metal atoms. A very first example for activating acetylenic proton under base and metal-free condition has been reported here. It gives a general method for synthesizing propargylic derivatives of cotarnine. An array of tetrahydro-isoquinolines alkaloids was synthesized by C(sp)-H bond activation of aromatic acetylenes with cotarnine at room temperature. A DFT based mechanism is proposed for
following reaction has been revealed.

Minh Hien Pham, Nicolas Auzeil, Anne Regazzetti, Daniel Scherman, Johanne Seguin, Nathalie Mignet, Daniel Dauzonne, Guy G Chabot (2016 Jul 29)
Metabolism of Flavone-8-acetic Acid in Mice.
Anticancer research : 3889-98

Résumé
Flavone-8-acetic acid (FAA) is a potent antivascular agent in mice but not in humans. Assuming that FAA was bioactivated in mice, we previously demonstrated that 6-OH-FAA was formed from FAA by mouse microsomes but not by human microsomes; its antivascular activity was 2.1- to 15.9-fold stronger than that of FAA, and its antivascular activity was mediated through the Ras homolog gene family (Rho) protein kinase A (RhoA) pathway. The present work aimed to study FAA metabolism in order to verify if 6-OH-FAA is formed in mice. Using synthesized standards and high-performance liquid chromatography (HPLC) coupled with ultraviolet (UV) detection and mass spectrometry (MS) analysis, we herein demonstrated, for the first time, that in vitro FAA and its monohydroxylated derivatives could directly undergo phase II metabolism forming glucuronides, and two FAA epoxides were mostly scavenged by NAC and GSH forming corresponding adducts. FAA was metabolized in mice. Several metabolites were formed, in particular 6-OHFAA. The antitumor activity of 6-OH-FAA in vivo is worthy of investigation.

Minh Hien Pham, Daniel Dauzonne, Guy G Chabot (2016 Feb 23)
Not flavone-8-acetic acid (FAA) but its murine metabolite 6-OH-FAA exhibits remarkable antivascular activities in vitro.
Anti-cancer drugs : 398-406 : DOI : 10.1097/CAD.0000000000000341

Résumé
Flavone-8-acetic acid (FAA) has been proved to be a potent vascular-disrupting agent in mice. Unfortunately, FAA did not produce any anticancer activity in clinical trials. Previously, we had reported that FAA is metabolized by mouse microsomes into six metabolites, whereas it was poorly metabolized by human microsomes, with fewer metabolites formed in lesser amounts. Especially, 6-OH-FAA was not formed by human microsomes. In this work, two major available metabolites, 4′-OH-FAA and 6-OH-FAA, were tested and compared with the parent compound FAA for their potential antivascular activities in vitro. The ability of the products to induce morphological changes, disrupt preformed capillaries of EA.hy926 endothelial cells and inhibit tubulin polymerization in vitro was assessed. The action mechanism was determined using the RhoA and Rac1 inhibitors. At 25 µg/ml, 6-OH-FAA induced morphological changes and membrane blebbing, whereas 300 µg/ml of FAA and 4′-OH-FAA slightly changed the morphology without inducing membrane blebbing. At 300 µg/ml, 6-OH-FAA produced morphological changes that were 2.1-6.9-fold greater than that produced by FAA and 4′-OH-FAA, an effect that was consistent with its much greater
inhibitory effect on tubulin polymerization compared with FAA and 4′-OH-FAA. 6-OH-FAA significantly disrupted the EA.hy926 cell capillaries. 6-OH-FAA activities were prevented in EA.hy926 cells pretreated with RhoA, but not Rac1, inhibitor. In this short communication we report for the first time that, in vitro, 6-OH-FAA, a mouse-specific FAA metabolite, exhibits significantly stronger antivascular activities compared with FAA and 4′-OH-FAA, which are mediated through the RhoA kinase pathway.


**Respiratory syncytial virus infection in macaques is not suppressed by intranasal sprays of pyrimidine biosynthesis inhibitors.**


**Résumé**

There is imperious need for efficient therapies against ubiquitous and life-threatening respiratory viruses, foremost among them being the human respiratory syncytial virus (hRSV). Several research groups who performed functional screens for broad-spectrum antivirals identified compounds targeting the de novo pyrimidine biosynthesis pathway. Despite their strong antiviral activity in vitro, whether such antimetabolites are effective in vivo remains highly controversial. Here, we evaluated two potent pyrimidine biosynthesis inhibitors developed in our laboratory, IPPA17-A04 and GAC50, in a model of mild hRSV-infection in cynomolgus macaques. In this model, hRSV replication is restricted to the epithelium of the upper respiratory tract, and is compatible with a topical treatment by intranasal sprays. The local administration of palivizumab, a neutralizing anti-hRSV antibody used in clinics, significantly reduced virus replication. In contrast, pyrimidine biosynthesis inhibitors did not show any inhibitory effect on hRSV growth when delivered topically as experimented in our model. Our results should help to better define the potential applications of this class of antimetabolites in the treatment of viral infections.

Vesela Kostova, Estelle Dransart, Michel Azoulay, Laura Brulle, Siiau-Kun Bai, Jean-Claude Florent, Ludger Johannes, Frédéric Schmidt (2015 Oct 29)

**Targeted Shiga toxin-drug conjugates prepared via Cu-free click chemistry.**

*Bioorganic & medicinal chemistry* : 7150-7 : [DOI : 10.1016/j.bmc.2015.10.010]

**Résumé**

The main drawback of the anticancer chemotherapy consists in the lack of drug selectivity causing severe side effects. The targeted drug delivery appears to be a very promising strategy for controlling the biodistribution of the cytotoxic agent only on malignant tissues by linking it to tumor-targeting moiety. Here we exploit the natural characteristics of Shiga
toxin B sub-unit (STxB) as targeting carrier on Gb3-positive cancer cells. Two cytotoxic conjugates STxB-doxorubicin (STxB-Doxo) and STxB-monomethyl auristatin F (STxB-MMAF) were synthesised using copper-free ‘click’ chemistry. Both conjugates were obtained in very high yield and demonstrated strong tumor inhibition activity in a nanomolar range on Gb3-positive cells.

Cristiane Salum, Fanny Schmidt, Patrick P Michel, Elaine Del-Bel, Rita Raisman-Vozari (2015 Sep 23)
**Signaling Mechanisms in the Nitric Oxide Donor- and Amphetamine-Induced Dopamine Release in Mesencephalic Primary Cultured Neurons.**
*Neurotoxicity research*: 92-104 : [DOI : 10.1007/s12640-015-9562-8]

**Résumé**

Previous research has shown that nitric oxide (NO) synthase inhibitors prevent rodents’ sensorimotor gating impairments induced by dopamine releasing drugs, such as amphetamine (Amph) and methylphenidate. The mechanisms of this effect have not been entirely understood. In the present work, we investigated some possible mechanisms by which the NO donor, NOC-12 (3-ethyl-3-(ethylaminoethyl)-1-hydroxy-2-oxo-1-triazene), influence spontaneous and Amph-induced dopamine release, using rat mesencephalic primary cultured neurons preparations. Our results showed that NOC-12 increased dopamine release in a concentration-dependent manner and potentiated the Amph-induced one. Dopamine release induced by NOC-12 was disrupted by N-acetyl-L-cystein (NAC—a free radical scavenger) and MK-801, a NMDA (N-methyl-D-aspartate) non-competitive antagonist, and was concentration dependently affected by oxadiazolo[4,3]quinoxalin-1-one, an inhibitor of the soluble guanylate cyclase (sGC). In contrast, dopamine released by Amph was facilitated by NAC and by MK-801 and not affected by nifedipine (a L-type-Ca(+2) channel blocker), which enhanced NOC-12-induced dopamine release. The present work demonstrates that DA release induced by NOC-12 is partially dependent on sGC and on NMDA activation, and is modulated by L-type Ca(+2) channel and the antioxidant NAC. This mechanism differs from the Amph-induced one, which appears not to depend on L-type Ca(+2) channel and seems to be facilitated by NMDA channel blocking and by NAC. These results suggest that Amph and NOC-12 induce dopamine release through complementary pathways, which may explain the potentiation of Amph-induced dopamine release by NOC-12. These findings contribute to understand the involvement of NO in dopamine-related neuropsychiatric and neurodegenerative diseases.

Ahmed Alouane, Raphaël Labruère, Thomas Le Saux, Frédéric Schmidt*, Ludovic Jullien* (2015 Jun 9)
**Self-immolative spacers: kinetic aspects, structure-property relationships, and applications.**
*Angewandte Chemie (International ed. in English)*: 7492-509 : [DOI : 10.1002/anie.201500088]
Résumé

Self-immolative spacers are covalent assemblies tailored to correlate the cleavage of two chemical bonds after activation of a protective part in a precursor: Upon stimulation, the protective moiety is removed, which generates a cascade of disassembling reactions leading to the temporally sequential release of smaller molecules. Originally introduced to overcome limitations for drug delivery, self-immolative spacers have gained wide interest in medicinal chemistry, analytical chemistry, and material science. For most applications, the kinetics of the disassembly of the activated self-immolative spacer governs functional properties. This Review addresses kinetic aspects of self-immolation. It provides information for selecting a particular self-immolative motif for a specific demand. Moreover, it should help researchers design kinetic experiments and fully exploit the rich perspectives of self-immolative spacers.

Claire Soudais, Fatoumata Samassa, Manal Sarkis, Lionel Le Bourhis, Stéphanie Bessoles, Didier Blanot, Mireille Hervé, Frédéric Schmidt, Dominique Mengin-Lecreulx, Olivier Lantz (2015 Apr 15)

In Vitro and In Vivo Analysis of the Gram-Negative Bacteria-Derived Riboflavin Precursor Derivatives Activating Mouse MAIT Cells.

Journal of immunology (Baltimore, Md. : 1950) : 4641-9 : DOI : 10.4049/jimmunol.1403224

Résumé

Mucosal-associated invariant T (MAIT) cells recognize microbial compounds presented by the MHC-related 1 (MR1) protein. Although riboflavin precursor derivatives from Gram-positive bacteria have been characterized, some level of ligand heterogeneity has been suggested through the analysis of the MAIT cell TCR repertoire in humans and differential reactivity of human MAIT cell clones according to the bacteria. In this study, using Gram-negative bacteria mutated for the riboflavin biosynthetic pathway, we show a strict correlation between the ability to synthesize the 5-amino-ribityl-uracil riboflavin precursor and to activate polyclonal and quasi-monoclonal mouse MAIT cells. To our knowledge, we show for the first time that the semipurified bacterial fraction and the synthetic ligand activate murine MAIT cells in vitro and in vivo. We describe new MR1 ligands that do not activate MAIT cells but compete with bacterial and synthetic compounds activating MAIT cells, providing the capacity to modulate MAIT cell activation. Through competition experiments, we show that the most active synthetic MAIT cell ligand displays the same functional avidity for MR1 as does the microbial compound. Altogether, these results show that most, if not all, MAIT cell ligands found in Escherichia coli are related to the riboflavin biosynthetic pathway and display very limited heterogeneity.

Cornélie Batisse, Estelle Dransart, Rafik Ait Sarkouh, Laura Brulle, Siau-Kun Bai, Sylvie Godefroy, Ludger Johannes, Frédéric Schmidt (2015 Apr 8)

A new delivery system for auristatin in STxB-drug conjugate therapy.

European journal of medicinal chemistry : 483-91 : DOI : 10.1016/j.ejmech.2015.03.047
Résumé

A key challenge in anticancer therapy is to gain control over the biodistribution of cytotoxic drugs. The most promising strategy consists in conjugating drugs to tumor-targeting carriers, thereby combining high cytotoxic activity and specific delivery. To target Gb3-positive cancer cells, we exploit the non-toxic B-subunit of Shiga toxin (STxB). Here, we have conjugated STxB to highly potent auristatin derivatives (MMA). A former linker was optimized to ensure proper drug-release upon reaching reducing environments in target cells, followed by a self-immolation step. Two conjugates were successfully obtained, and in vitro assays demonstrated the potential of this targeting system for the selective elimination of Gb3-positive tumors.

Année de publication : 2014

Janos Sapi, Frédéric Schmidt, Luc Van Hijfte, Pascal George (2014 Dec 6)
Interfacing chemical biology and drug discovery: report from the 50th International Conference on Medicinal Chemistry of the SCT (French Medicinal Chemistry Society), July 2-4, 2014, Rouen, France.
ACS chemical biology : 2702-7 : DOI : 10.1021/cb5009469

Résumé

Frédéric Schmidt, Pascal George, Janos Sapi (2014 Apr 19)
ACS chemical biology : 849-52 : DOI : 10.1021/cb500173s

Résumé