

Proceedings | *Resumos*

***Challenging organic syntheses inspired by nature from
natural products chemistry to drug discovery***

COST Action CM1407

Meeting dedicated to Early Career
Investigators



COST Action CM1407

Ação COST CM1407

18-19 February | 18-19 Fevereiro

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18 February | 18 de Fevereiro

C.01 - Natural Products as Carbonic Anhydrase Inhibitors

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Abstract / Resumo da Comunicação

Carbonic anhydrases (CAs, EC 4.2.1.1) catalyze the reversible conversion of CO₂ to HCO₃⁻ and proton in all living organisms, including Archaea, Bacteria, and Eukarya [1]. Seven genetically unrelated families of such enzymes have been identified so far, which result from a convergent evolution process. CA isozymes have acquired great interest for the design of inhibitors (CAIs) or activators (CAAs) with biomedical applications [1]. For more than 70 years synthetic CAIs of the sulfonamide type have been used in clinical practice as diuretics and systemic antiglaucoma drugs and many other synthetic CAIs belonging to a variety of chemotypes have been evaluated in the treatment of a wealth of other diseases, such as obesity, tumors, inflammation and infections [2]. Recent studies using natural product libraries and isolated constituents from natural sources provided novel chemotypes possessing CA inhibition activities [3-5]. It should be noted that natural products comprising zinc binding groups in their structure are rare and the relatively few natural products explored to date showed innovative mechanisms of action. The natural product chemical space allowed the discovery of novel drug-like derivatives and has been offering rich opportunities in the search for new, more efficient CAIs.

References:

- [1] Supuran CT (2008). *Nat Rev Drug Discov* 7: 168-81.
- [2] Alterio V, Di Fiore A, D'Ambrosio K, Supuran CT, De Simone G (2012). *Chem Rev* 8: 4421-68
- [3] Maresca A, Temperini C, Vu H, Pham NB, Poulsen SA, Scozzafava A, Quinn RJ, Supuran CT (2009). *J Am Chem Soc* 131: 3057-62.
- [4] Carta F, Temperini C, Innocenti A, Scozzafava A, Kaila K, Supuran CT (2010) *J Med Chem* 53: 5511-22.
- [5] Karioti A, Carta F, Supuran CT (2016). *Curr Pharm Des* 22:1570-91.

C.02 - Countercurrent separation of biologically active natural products: the case of acidic cannabinoids

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Abstract / Resumo da Comunicação

Countercurrent separation techniques have an increasingly pivotal role in the isolation of natural products [1]. Among them, centrifugal partition chromatography (CPC) has been the technique of choice for numerous applications, including the isolation of cannabinoids from the plant material of *Cannabis sativa* L. [2]. These terpenophenolic compounds are associated with a considerable therapeutic potential, as revealed by on-going research [3]. Cannabidiol (CBD) and its biogenetic precursor cannabidiolic acid (CBDA) represent the major non-psychoactive cannabinoids in fibre-type *C. sativa* (hemp), possessing numerous pharmacological properties [3,4].

This study reports an effective strategy for the rapid isolation of the major cannabinoids originally present in *C. sativa*, based on their ionisable character. The selective separation of cannabidiolic acid (CBDA), as a paradigm, from the hemp extract obtained through supercritical fluid extraction (SFE) was performed by means of CPC in the pH-zone-refining mode [5]. The fractionation was carried out using a biphasic solvent system, consisted of n-hexane/ethyl acetate/ethanol/water 8:2:5:5 (v/v/v/v). Trifluoroacetic acid was used as retainer in the organic stationary phase, while triethylamine was added as eluter in the aqueous mobile phase. The most promising fraction obtained by CPC was subsequently submitted to liquid-liquid extraction, in order to recover CBDA from the salt form as well as for further purification. Following this two-step procedure, more than 45% (w/w) of CBDA was totally recovered with higher purity compared to previously reported methodologies [2], i.e. greater than 95%, as revealed by HPLC-PDA analysis. Taking into account the easy scale-up of CPC, this methodology can be efficiently transferred to industrial scale and could be readily adaptable for the isolation of other acidic cannabinoids.

Keywords: Cannabidiolic acid (CBDA), cannabidiol (CBD), phytocannabinoids, *Cannabis sativa* L., preparative isolation

References:

- [1] Friesen JB, McAlpine JB, Chen SN, Pauli GF. *J Nat Prod* 2015; 78: 1765–1796.
- [2] Hazekamp A, Simons R, Peltenburg-Looman A, Sengers M, van Zweden R, Verpoorte R. *J Liq Chromatogr Relat Technol* 2004; 27: 2421–2439.
- [3] Izzo AA, Borrelli F, Capasso R, Di Marzo V, Mechoulam R. *Trends Pharmacol Sci* 2009; 30: 515–527.
- [4] Morales P, Reggio PH, Jagerovic N. *Front Pharmacol* 2017; 8: 422.
- [5] Ito Y. *J Chromatogr A* 2013; 1271: 71–85.

C.03 - Self-assembling Releasable Thiocolchicine-Diphenylbutenylaniline Conjugates

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Abstract / Resumo da Comunicação

In recent years the use of prodrugs has become an important tool to improve the pharmacokinetic properties of drugs and overcome a range of different issues, including low bioavailability due to poor absorption and degradation by protective mechanisms after reaching the target site.

In this wide field, we are particularly interested in the synthesis of anticancer drug-conjugates that spontaneously form nanoparticles in aqueous media.

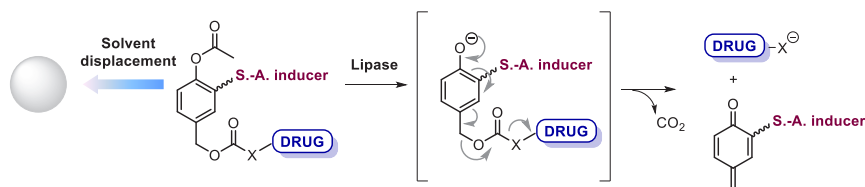
We obtained several constructs using squalene-1-3 or 4-(1,2-diphenylbut-1-en-yl)aniline-4 as self-assembling inducers and a disulfide-containing linker able to release the drug inside the cell.

To improve the cleavage of the bonds forming the conjugate we introduced a different linker, inspired from known self-immolative ones, able to trigger the release of the thiocolchicine moiety in the presence of a lipase.

The formation of nanoparticles was confirmed by DLS, ATM and fluorescence microscopy, and their antiproliferative activity has been proved on two human cancer cell lines.

References:

- [1]. Borrelli S, Christodoulou M. S, Passarella D, et al (2014). Eur J Med Chem 85: 179-190.
- [2]. Borrelli S, Cartelli D, Passarella D, et al (2015). ChemPlusChem 80: 47-49.
- [3]. Fumagalli G, Stella B, Passarella D, et al (2017). ACS Med Chem Lett 8: 953-957.
- [4]. Fumagalli G, Christodoulou M. S, Passarella D, et al (2017). Org Biomol Chem 15: 1106-1109.



C.04 - The potential of c-Src inhibitors Si306 and pro-Si306 for suppressing invasion and overcoming multidrug resistance in glioblastoma

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Abstract / Resumo da Comunicação

Glioblastoma multiforme (GBM) are the most frequent and aggressive (WHO grade IV) brain tumors in adults. GBM have high expression of c-Src tyrosine kinase involved in survival, migration and invasiveness of tumor cells. Thus, c-Src emerged as a potential target for GBM therapy.

Cytotoxicity of c-Src inhibitors pyrazolo[3,4-d]pyrimidines, Si306 and its prodrug pro-Si306, was investigated in human GBM cell line U87 and its multidrug resistant (MDR) counterpart U87-TxR by MTT assay. Anti-migratory and anti-invasive effects of Si306 and pro-Si306 were assessed by wound healing, gelatin degradation and transwell invasion assays. The effect of c-Src inhibitors on P-glycoprotein (P-gp) activity in U87-TxR cells was analyzed by flow cytometry. Their ability to reverse paclitaxel resistance in MDR cells was also assessed. Zebrafish model was used to evaluate anti-invasive potential of pro-Si306 on U87 xenografts in vivo.

Novel c-Src inhibitors were significantly more efficient in cell growth inhibition compared to the well-known tyrosine kinase inhibitor dasatinib. The efficacy of Si306 and pro-Si306 was not affected by the MDR phenotype.

Migratory potential of U87 and U87-TxR cells was significantly decreased by both inhibitors. The ability of cells to degrade the matrix and invade through basement membrane was also significantly impaired upon treatment with Si306 and its prodrug. Assessment of intracellular accumulation of fluorescent P-gp substrate showed that both compounds inhibited P-gp activity in U87-TxR cells. Si306 and pro-Si306 also enhanced the paclitaxel efficacy in resistant glioblastoma. In vivo pro-Si306 showed anti-invasive effect against U87 xenografts in zebrafish model.

Considering their ability to suppress migration and invasion and overcome MDR, Si306 and pro-Si306 could be considered in GBM treatment alone or in combination with other chemotherapeutics.

C.05 - Identification of a new class of Tyrosinase Inhibitors via Computational Studies, Synthesis and Biological Evaluation

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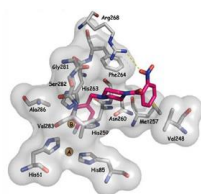
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Abstract / Resumo da Comunicação

Tyrosinases (Tys, EC 1.14.18.1) are metalloenzymes belonging to the “type-3 copper” proteins family and possessing a conserved six histidine residues coordinated by two copper ions (CuA and CuB). They exist in all life domains and are involved in the mammal biosynthesis of melanin, important pigment which perform a crucial role in skin, hair, eyes pigmentation and in skin protection against UV radiations damages. However, an excessive production of melanin can cause serious skin diseases. Thus, Tys inhibition is an established strategy to avoid these side effects and the development of TyIs gained high interest in the therapy of skin pathologies, as well as, in dermocosmetic treatments. To date several TyIs, such as hydroquinone, arbutin and kojic acid were developed, but unfortunately they showed relevant human toxicity¹. For this reason, there is still an urgent needing for new derivatives with better pharmacological characteristics. A combination of docking and crystallographic studies on mushroom (e.g. *Agaricus bisporus*, TyM) and bacterial (e.g. *Bacillus megaterium*, TyBm) Tys respectively allowed us to design a new class of derivatives bearing 4-fluorobenzylpiperidine and 4-fluorobenzylpiperazine portions, many of them possess higher efficacy than the reference compound kojic acid (IC₅₀ = 17.76 µM)^{2,3}. The crystal structures of TyBm with the most promising inhibitors were solved, confirming the binding poses hypothesized by in silico studies. The 4'-fluorobenzyl moiety is located between the two copper ions, with the aromatic ring stabilized through stacking interactions within hydrophobic wall of catalytic pocket and could exert a crucial role in controlling the inhibitory effect. The so planned compounds were then synthesized and their biological activity was evaluated against TyM, thus identifying a class of potent inhibitors with various structural modifications (IC₅₀ ≤ 0.96 µM).

References:

- [1]. Pillaiyar T, Manickam M, Namasivayam V (2017). *J Enzyme Inhib Med Chem* 32: 403-425.
- [2]. Ferro S, De Luca L, Germano M P, Buemi M R, Ielo L, Certo G, Kanteev M, Fishman A, Rapisarda A, Gitto R (2017) *Eur J Med Chem* 125: 992-1001.
- [3]. Ferro S, Deri B, Germano M P, Gitto R, Ielo L, Buemi M R, Certo G, Vittorio S, Rapisarda A, Pazy Y, Fishman A, De Luca L (2018). *J Med Chem* 61: 3908-3917



C.06 - Discovering drugs to overcome anti-cancer drug resistance

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Abstract / Resumo da Comunicação

Intrinsic and acquired resistance to conventional and targeted chemotherapeutics is the fundamental reason for treatment failure for many cancer patients. The identification of molecular mechanisms involved in drug resistance or sensitization to targeted therapy is of enormous clinical importance. Moreover, critical transcription factors such as forkhead box O (FOXO) proteins have been shown to mediate the action of multiple anti-cancer drugs.

The Link lab has discovered a novel mechanism of drug resistance mediated by TRIB2 (Tribbles Pseudokinase 2) (1). TRIB2 potentiates AKT activation leading to FOXO cytoplasmic sequestration and consequently inactivation (2). Recent studies have also shown that TRIB2 might have residual catalytic activity and function as a kinase (3). These exciting data emphasizes the interest on TRIB2 as a druggable target. Our lab is taking several approaches to identify pharmacological means to manipulate TRIB2 activity. In this context, we generated RNA-Seq data using two isogenic cell lines where we modulated TRIB2 expression levels. This approach allowed us to retrieve a list of genes that are differentially regulated upon TRIB2 over-expression. Using the Connectivity Map bioinformatic tool (c-MAP; Broad institute) we were able to produce a list of select bioactive chemical compounds, in particular FDA approved drugs, capable of producing an inverse gene expression profile. cMAP is a large database of gene expression data modulated by treatment with drugs (small molecules) in four human cell lines HL60 (promyelocytic leukemia), MCF7 (human breast adenocarcinoma), PC3 (prostate cancer) and SKMEL5 (skin melanoma) and contains 6100 treatment experiments and 1309 unique drugs (many are FDA approved) (4). We obtained a list of candidate drugs (Harmine; Piperlongumine; Irinotecan and LM-1685) by ruling out enrichment scores with P-values greater than 0.05. Preliminary data from our lab showed that Harmine is able to induce FOXO-GFP translocation to the nucleus to the same extent as the nuclear export inhibitor Leptomycin B (inhibitor of Exportin1) (5) The data obtained with this project will be particularly relevant as a new therapeutic opportunity for patients that over-express TRIB2 and that are more prone to develop resistance.

References:

1. Zanella, F., Renner, O., García, B., Callejas, S., Dopazo, A., Peregrina, S., Carnero, A., and Link, W. (2010) Human TRIB2 is a repressor of FOXO that contributes to the malignant phenotype of melanoma cells. *Oncogene*. 29, 2973–2982
2. Hill, R., Madureira, P. A., Ferreira, B. I., Baptista, I., Machado, S., Colaço, L., Santos, Dos, M., Liu, N., Dopazo, A., Ugurel, S., Adrienn, A., Kiss-Toth, E., Isbilen, M., Gure, A. O., and Link, W. (2017) TRIB2 confers resistance to anti-cancer therapy by activating the serine/threonine protein kinase AKT. *Nat Commun.* 8, 14687
3. Bailey, F. P., Byrne, D. P., Oruganty, K., Eysers, C. E., Novotny, C. J., Shokat, K. M., Kannan, N., and Eysers, P. A. (2015) The Tribbles 2 (TRB2) pseudokinase binds to ATP and autophosphorylates in a metal-independent manner. *Biochem J*. 467, 47–62
4. Lamb, J., Crawford, E. D., Peck, D., Modell, J. W., Blat, I. C., Wrobel, M. J., Lerner, J., Brunet, J.-P., Subramanian, A., Ross, K. N., Reich, M., Hieronymus, H., Wei, G., Armstrong, S. A., Haggarty, S. J., Clemons, P. A., Wei, R., Carr, S. A., Lander, E. S., and Golub, T. R. (2006) The Connectivity Map: using gene-expression signatures to connect small molecules, genes, and disease. *Science*. 313, 1929–1935
5. Kudo, N., Matsumori, N., Taoka, H., Fujiwara, D., Schreiner, E. P., Wolff, B., Yoshida, M., and Horinouchi, S. (1999) Leptomycin B inactivates CRM1/exportin 1 by covalent modification at a cysteine residue in the central conserved region. *Proc Natl Acad Sci USA*. 96, 9112–9117

C.07 - Largazole, a promising scaffold for HDAC inhibitors

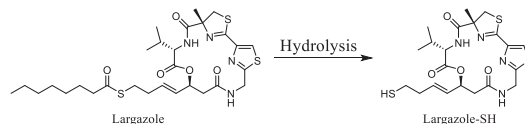
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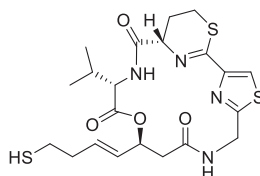
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Abstract / Resumo da Comunicação

Largazole is a marine cyanobacterium metabolite (Symploca genus) isolated by Luesch and co-workers, with novel chemical scaffold. It consists of a strained 16-membered cyclic depsipeptide bearing a thioester moiety that, in physiological condition, is hydrolyzed leaving the active HDAC inhibitor Largazole-SH.



Largazole showed a sub-nanomolar inhibition potency with high selectivity for class I HDAC. Furthermore, its selective cytotoxicity for cancer cell lines (e.g.: MDA-MB-231 GI₅₀ = 7.7 nM) compared to non-transformed cells (e.g.: NMuMG GI₅₀ = 122 nM),^{1,2} pushes us towards the search for new derivatives with greater potency and improved HDAC isoform selectivity.



5,6-dihydro-4H-1,3-thiazine derivative

The synthesis of Largazole, along with its chemical challenges, is presented, as well as its adaptation for the preparation of 5,6-dihydro-4H-1,3-thiazine derivative which, among the planned derivatives, is of great interest for its anticipated HDAC isoform selectivity.

References:

- [1]. Kim B, Park H, Salvador LA, Serrano PE, Kwan JC, Zeller SL, Chen QY, Ryu S, Liu Y, Byeon S, Luesch H, Hong J. *Bioorg Med Chem Lett*. 2014 Aug 15;24(16):3728-31
- [2]. Taori K, Paul V.J. and Luesch H J. *Am. Chem. Soc.*, 2008, 130 (6), pp 1806–1807

C.08 - A General Approach for the Enantioselective Total Synthesis of cis-Decahydroquinoline Alkaloids

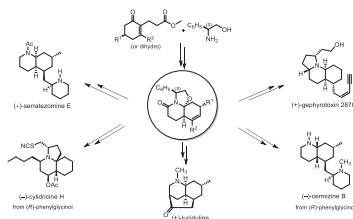
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Abstract / Resumo da Comunicação

The decahydroquinoline (DHQ) ring system is present in a large number of biologically active natural products. Unlike most alkaloid types, DHQ-containing alkaloids occur not only in plant species (e.g., *Lycopodium* species: cernicene B, serratezomine E, and luciduline)¹ but also in marine (e.g., tunicates and flatworms: cylindricines and lepadins) and other terrestrial (e.g., amphibians and arthropods: pumiliotoxin C and gephyrotoxin 287C)² organisms. The scarcity of these natural products and their interesting biological activities make these alkaloids attractive synthetic targets.

We will present our synthetic studies on the enantioselective synthesis of structurally diverse cis-DHQ alkaloids, using phenylglycinol-derived tricyclic lactams as chiral scaffolds.



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References:

- [1]. a) Pinto A, Griera R, Molins E, Fernández I, Bosch J, Amat M (2017). *Org Lett* 19: 1714-1717; b) Pinto A, Picciché M, Griera R, Molins E, Bosch J, Amat M (2018). *J Org Chem* 83: 8364–8375.
- [2]. Picciché M, Pinto A, Griera R, Bosch J, Amat M (2017). *Org Lett* 19: 6654-6657.

C.09 - Uncovering the cyanobacterial chemical diversity

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Abstract / Resumo da Comunicação

Cyanobacteria represent an important group of photosynthetic bacteria with diverse morphologies and ecological niches. This remarkable ecophysiological diversity is also reflected in a wide variety of unique cyanobacterial secondary metabolites, making them an interesting resource for natural products drug discovery [1]. Brentuximab vedotin 63 (Adcetris™), a FDA approved anticancer drug, is the most successful example of this [2]. Currently, other 17 compounds are under clinical trial [3].

A valuable and underexplored natural resource that can underpin the discovery of promising compounds can be found within The Culture Collection of cyanobacteria (LEGEcc) at Blue Biotechnology and Ecotoxicology at CIIMAR. LEGEc comprises more than 350 different cyanobacterial strains, collected in different ecosystems and locations [4].

Herein, early stage results of CYANCAN project will be presented. This project has as main goal the discovery of new anticancer compounds derived from cyanobacteria. The isolation of the compounds will be performed by the classical bioassay-guided fractionation, and by modern genome-guided natural products discovery. As such, the genome-isotopologue strategy will be key to unveil cryptic compounds and give insights into their biosynthesis. In order to find successful anticancer lead candidates the CYANCAN project will employ physiologically relevant cancer screening assays (3D tumor spheroids), and in a later stage study the mechanisms of action, as well as, address the main cancer multidrug resistance mechanism.

References:

- [1]. Leão PN, Engene N, Antunes A, Gerwick GW, Vasconcelos V (2012) Nat Prod Rep 29: 372.
- [2]. Jaspars M, De Pascale D, Andersen JH, Reyes F, Crawford AD, Ianora A (2016) J Mar Biol Assoc United Kingdom 96: 151–158.
- [3]. <http://marinepharmacology.midwestern.edu/clinPipeline.htm>
- [4] Ramos V, Morais J, Castelo-Branco R, Pinheiro A, Martins J, Regueiras A, Pereira AL, Lopes VR, Frazão B, Gomes D, Moreira C, Costa MS, Brûle S, Faustino S, Martins R, Saker M, Osswald J, Leão PN, Vasconcelos VM (2018) J Appl Phycol 1–15.

C.10 - Insight into the symbiotic chemical communication of Algae and Bacteria: Thallusin and Dedicated Analogues

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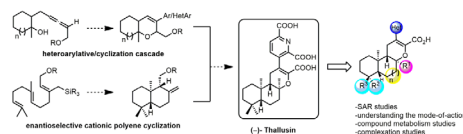
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Abstract / Resumo da Comunicação

Thallusin was the first compound identified to induce thallus differentiation in macroalgae. This intriguing chemical mediator is produced by bacteria that socialize with algae in their natural habitat. Thallusin exemplifies a fundamental symbiotic chemical communication between macroalgae and epiphytic bacteria in the marine environment. However, its general impact on the underlying microbial communities and its chemical mode-of-action remains to be clarified. Therefore we propose a multifaceted research program to study the morphogenesis-inducing mechanism of algae by chemical morphogen. The proposed project is focus at four different objectives. 1) methodologies (heteroarylative/cyclization cascade and enantioselective, cationic polyene cyclization), that allows the efficient and concise synthesis of thallusin. This will be achieved by transition metal catalysis and chiral Bronsted acid respectively based on good literature precedent. 2) This objective is in progress. 2) development of novel, concise, scalable, and efficient asymmetric synthesis of (–)-thallusin and its analogues. This will be accomplished by enantioselective polyene cyclization and heteroarylative/cyclization cascade as key steps. 3) structure-activity-relationships studies and 4) understanding the mode-of-action of thallusin in algae. 3 The labeled tool compounds and dye conjugates will be synthesized from promising candidates that will allow tracing the metabolism and degradation of thallusin in algae. The results that will be obtained during the course of our research will have the potential capacity to enlighten the cross-kingdom cell-to-cell chemical signaling and will provide room for further studies at the fundamental and applied levels in green algae and symbiotic bacteria association.

References:

- [1]. Matsuo Y, Imagawa H, Nishizawa M, Shizuri Y (2005). Science 307: 1598.
- [2]. Zimmer R, Dinesh C. U, Nandan E, Khan F. A (2000). Chem Rev 100: 3067. Surendra K, Rajendar G, Corey E (2014). J Am Chem Soc 136: 642.
- [3]. The objectives 3 and 4 will be completed in collaborations with Dr. Thomas Wichard



C.11 - New functionalized flavonoids as potential anticancer agents

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Abstract / Resumo da Comunicação

In the last decades, the search of new and more efficient drugs has been one of the biggest challenges into the organic chemistry. From this point of view, Natural Products have had an important role in drugs discovery due to the great variety of biological activities and uses that have shown. We are focus in Flavonoids that are a big family of naturally occurring polyphenolic compounds which have a vast array of interesting properties among which stand out the anticancer activity. Therefore, these are considered privileged structures and excellent candidates for the development of new therapeutic agents.

Based on previous researches based on chalcones and other flavonoids both natural and synthetic, our objective is to generate a library of new triazolic chalcones by modifying the substituents on the aromatic rings in order to carried out a 1,3-dipolar cycloaddition between azides and terminal alkynes [1] (Figure 1). The new synthetic products will be tested against human cancer cell lines to study their anticancer potential.

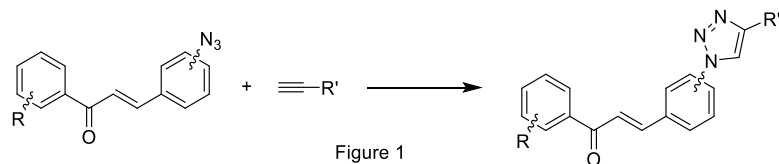
In this communication, the results of the synthesis and the studies carried out about the potential biological activity of these novel products, not described above in the scientific literature, will be discussed.

Acknowledgement:

This research was supported by the Spanish MINECO (CTQ2014-56362-C2-1-P), co-financed by the European Regional Development Fund (ERDF).

References:

- [1]. (a) Burmistrova O, Marrero MT, Estévez S, Welsch I, Brouard I, Quintana J, Estévez F (2014) Eur. J. Med. Chem. 84: 30-41. (b) Estévez-Sarmiento F, Said M, Brouard I, León F, García C, Quintana J, Estévez F (2017) Bioorg. Med. Chem. 25: 6060-6070.



C.12 - Synergistic inhibition of the Hedgehog pathway by newly designed Smo and Gli antagonists bearing the isoflavone scaffold

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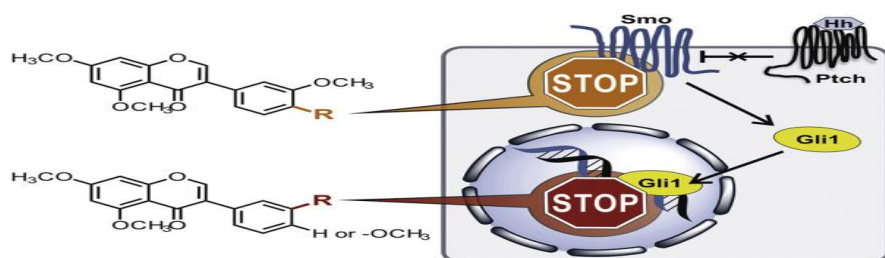
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Abstract / Resumo da Comunicação

Hedgehog (Hh) signaling has emerged in recent years as a druggable target for anticancer therapy.[1] Its aberrant activation, occurring either by ligand-dependent or -independent mechanisms, has been observed in many tumors. Thus, small molecules able to block the pathway at the upstream receptor Smoothened (Smo) or the downstream effector Gli1 have emerged as valuable anticancer agents. [2] We previously identified Glabrescione B (GlaB), an isoflavone naturally found in the seeds of *Derris glabrescens* (Leguminosae), as a novel small molecule that proved to be able to bind Gli1ZF and interfere with its interaction with DNA.[3] We provided the total synthesis of GlaB which foresees a mild and cost effective three-step protocol based on the deoxybenzoin.[4] This synthetic strategy allowed us the design and preparation of a small-size focused library of isoflavones bearing different substitutions at the ring B to elucidate the structure-activity relationships and the molecular mechanism behind the Hh signaling modulation. Target preference has been achieved by the introduction of specific bulky substitutions at meta position (targeting GLI1) or para position (targeting SMO) of the isoflavone's ring B.[4] Interestingly, the combined administration of two different isoflavones behaving as SMO and GLI1 antagonists, respectively, in primary MB cells has shown synergistic Hh inhibition at doses that are around 20-fold lower than individual compound doses, thus leading the way to further and innovative combination therapy for the treatment of Hh-dependent tumors.

References:

- [1]. Ruiz i Altaba A, Sanchez P, Dahmane N (2002) Nat. Rev. Canc. 2(5): 361-372.
 [2]. Ghirga F, Mori M and Infante P (2018) Bioorganic Med. Chem. Lett. 3131-3140.
 [3]. Infante P, Mori M, Alfonsi R, Ghirga F, Aiello F, Toscano S, Ingallina C, Siler M, Cucchi D, Po A, Miele E, D'Amico D, Canettieri G, De Smaele E, Ferretti E, Screpanti I, Uccello Barretta G, Botta M, Botta B, Gulino A, Di Marcotullio L (2015) EMBO J. 34: 200-217.
 [4]. Berardozi S, Bernardi F, Infante P, Ingallina C, Toscano S, De Paolis E, Alfonsi R, Caimano M, Botta B, Mori M, Di Marcotullio L, Ghirga F (2018) Eur J Med Chem. 156:554-562.



C.13 - Discovery of N-Phenylpyrrolamides as ATP-competitive human DNA topoisomerase II inhibitors with potential anticancer activity

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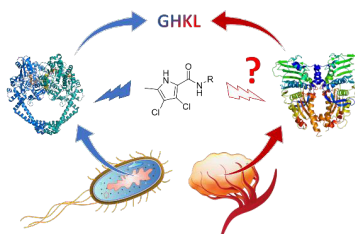
Abstract / Resumo da Comunicação

Human DNA-topoisomerase II is an ATP-dependent enzyme that plays vital roles in DNA transcription, replication and chromosome segregation and therefore represents an attractive target in anticancer drug discovery.¹ Because of the presence of GHKL ATPase domain, DNA topoisomerase II belongs to the same protein superfamily as bacterial DNA Gyrase, Hsp90, histidine kinase and MutL proteins.² Based on this fact we used the biochemical screening of existing ATP-competitive bacterial DNA Gyrase inhibitors as a starting point in discovery of new human DNA-topoisomerase inhibitors.

There is a library of about 1000 ATP-competitive DNA-gyrase inhibitors at University of Ljubljana, Faculty of Pharmacy, that is a product of extensive work of our research group on preparation of novel antibacterial agents.³⁻⁴ The library is based on the structure of marine alkaloid oroidin, isolated from Agelas sponges, which shows antibacterial activity. Initial screening of approximately 100 bacterial DNA-gyrase inhibitors resulted in identification of 12 hit compounds, 9 of which contained a common N-phenylpyrrolamide scaffold. Structure-based optimization of newly discovered hit compounds led to synthesis of new series of human DNA topoisomerase II inhibitors. New inhibitors are smaller molecules compared to the original hits, which gives them an improved potential for optimization. Cytotoxic activity of novel inhibitors was tested on MCF-7 and HepG2 cancer cell lines. Best compounds of the series show activity comparable to one of marketed drug etoposide, and further development is taking place to improve the on-target and cytotoxic activities of this type of compounds.

References:

- 1) Nitiss JL. DNA topoisomerase II and its growing repertoire of biological functions. *Nat Rev Cancer*. 2009; 9:327.
- 2) Dutta R, Inouye M. GHKL, an emergent ATPase/kinase superfamily. *Trends Biochem Sci*. 2000; 25(1):24-28.
- 3) Zidar N, Tomašič T, Macut H, et al. New N-phenyl-4,5-dibromopyrrolamides and N-Phenylindolamides as ATPase inhibitors of DNA gyrase. *Eur J Med Chem*. 2016; 117:197-211.
- 4) Durcik M, Tammela P, Barancokova M, et al. Synthesis and Evaluation of N-Phenylpyrrolamides as DNA Gyrase B Inhibitors. *ChemMedChem*. 2018; 13(2):186-198.



C.14 - Synthesis of novel heterocyclic compounds via electrophilic cyclization reactions

Arif Kivrak

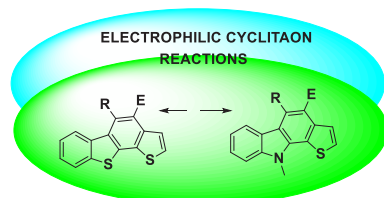
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Abstract / Resumo da Comunicação

Polyhetero-aromatic compounds like carbazoles, indoles, benzothiophenes have emerged as central candidates for pharmaceutical applications since they show remarkable analgesic, anti-inflammatory, anti-bacterial antiparasitic, antitussive, hypoglycemic, antitumor and/or anticancer activities [1,2]. A variety of synthetic methods have been used for the synthesis of poly-heteroaromatic compounds and they have also been used to treat different diseases for many years. On the other hand, there are a few studies for thienocarbazole and thienobenzothiophene derivatives which may have critical biological properties [3]. In the present study, we developed a novel methodologies for the synthesis of potentially biologically active new thienobenzothiophene and thienocarbazole derivatives by using electrophilic cyclization reactions starting from o-iodothiobenzonitrile and 2-iodoaniline. The detail results will be presented at meeting.

References:

- [1]. Gilchrist T. L. *Heterocyclic Chemistry*, Longman publications, London 1992, pp. 175-319.
- [2]. John AJ, Keith M. *Heterocyclic Chemistry*, Wiley, 5th Edition 2010; pp. 433-445.
- [3] Ji, X; Su, M; Wang, J; Deng, G; Deng, S; Li, Z; Tang, C; Li, J; Li, J; Zhao, L; Jiang, H; Liu, H Design, synthesis and biological evaluation of hetero-aromatic moieties substituted pyrrole-2-carbonitrile derivatives as dipeptidyl peptidase IV inhibitors. *Eur J Med Chem* 2014; 75:111-22.



C.15 - Targeting Hedgehog pathway in CSCs treatment: synthesis of a new inhibitor

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Abstract / Resumo da Comunicação

Cancer stem cells (CSCs) are a subpopulation of cancer cells, characterized by the self-renewal properties typical of all the stem cells, as well as by the ability to generate heterogeneous cancer cells. These peculiarities are the basis of the drug resistance mechanism induced by CSCs, responsible of the relapse in different tumors. In fact, CSCs are unaffected by traditional chemotherapeutic agents.[1]

Thus, the search for novel therapies able to target both differentiated tumor cells and CSCs, are still of primary importance.

In this field, Hedgehog (Hh) signalling pathway emerged in the last years as a promising target, because its aberrant activation seems to be involved in carcinogenesis and in the maintenance of CSCs. [2]

Therefore, we propose a new scaffold, rationally designed taking inspiration by nature and in particular by withanolides. Withanolides are a wide family of plant-derived products; some of them, as Withaferin A and some of its simplified analogs,[3] recently proved to inhibit the Hh pathway, targeting the so called “smoothened protein” (SMO), involved in Hh pathway abnormal activation.

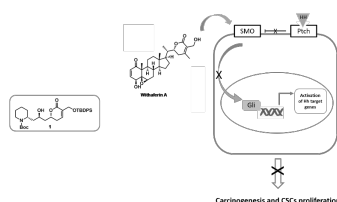
So far, an advanced intermediate 1 has been synthesized in our group. Once obtained the final compound, it will be tested to evaluate its ability to target the SMO protein, preventing in this way CSCs proliferation.

References:

[1]. Kwon H Y et al. (2018) Stem Cells Int. 2018: 5416923.

[2] Cain J E et al. (2015) Cancers, 7: 1554–1585.

[3]. Waldmann H et al. (2015) Angew. Chem. Int. Ed. 54: 5596-5602.



C.16 - *Plectranthus* species: anticancer lead molecules search

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Abstract / Resumo da Comunicação

Natural Products and derivatives are a rich source of anti-cancer drugs [1]. Plants from *Plectranthus* genus (Lamiaceae) have demonstrated several uses, including the treatment of various infections [2]. *Plectranthus* spp. are known to be a good source of bioactive compounds namely, abietane-type diterpenoids as common secondary metabolites with reported biological activities [3].

The objective of this study was to evaluate the biological activity of sixteen *Plectranthus* spp. acetonic extracts and identify the bioactive compounds in the most active extracts. *P. mutabilis* had the highest extraction yield (30.03%, dry weight % w/w). All extracts were screened for their antimicrobial activity against Gram positive (*Staphylococcus aureus* and *Enterococcus faecalis*) Gram negative (*Pseudomonas aeruginosa* and *Escherichia coli*) bacteria and two yeast strains (*Candida albicans* and *Saccharomyces cerevisiae*). Nine extracts were active against *C. albicans* and two against *S. aureus* using the well diffusion test. The most active extracts *P. ramosior* and *P. mutabilis* were tested through the microdilution method (*P. ramosior* extract: MIC value of 15.625 µg/mL against *S. aureus* and MIC value of 3.91 µg/mL in MRSA strain). All the extracts showed antioxidant activity by the DPPH assay and *P. ramosior* (36.24%) and *P. mutabilis* (46.14%) had the highest percentage scavenging activity. Furthermore, the antitumor activity of the five most cytotoxic extracts was explored in different cancer cell lines: HCT116, MCF-7 and NCI-H460. *P. ramosior* was the most cytotoxic in all the cancer cell lines (HCT116 = 3.45±0.35, MCF-7 = 2.90 ±0.10, NCI-H460 = 3.00±0.10).

In conclusion, *P. ramosior* was the most cytotoxic extract, and the chromatographic methodologies resulted in the isolation of the compound 7 α -acetoxy-6 β -hydroxyroyleanone. The bioactive compound is the potential responsible for the cytotoxic activity of the extract, which was confirmed by the studies in human cell lines (NCI-H460, NCI-H460/R and MRC-5).

References

[1]. Seca M. L and Pinto G. A (2018). Int. J. Mol. Sci. 19: 263

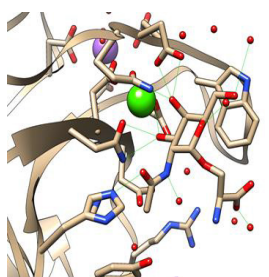
[2]. Lukhoba C, Simmonds M, Paton A (2006). Journal of Ethnopharmacology 103: 1–24.

[3]. Burmistrova O Fatima, M, Rijo P, Quintana J, Bermejo J, Estevez F (2013). Journal of Natural Products 10-1021

19 February | 19 de Fevereiro

C.01 - Protein-carbohydrate interaction: the sweet language of natureAdele Gabba^a, Gabriel Birrane^b, Paul V. Murphy^a^a School of Chemistry, National University of Ireland Galway^b Experimental Medicine, Harvard Medical School**Abstract / Resumo da Comunicação**

Carbohydrates are the most abundant natural products. Their complexity makes them perfect building block of a sophisticated language that can convey the broadest set of information. Each letter of this natural alphabet differs, not only from the stereocenters, which constitute the ring, but also from ring size, linkage position, branching and functionalisation. Apart from their role in metabolism, they are indispensable components of every cell surface, where they are involved in vital cellular recognition processes. Advances in the functional understanding of carbohydrate-protein interactions, have enabled the development of a new class of small-molecule drugs, known as glycomimetics. These compounds simultaneously mimic the bioactive function of carbohydrates while addressing the drawbacks of them, mainly exploiting multivalency effect.



Lectins are the main class of protein which recognize glycans. A member of the C-type lectin family is CLEC10A (h-MGL, CD301), an endocytic receptor located on the surface of some immune system cells, mainly dendritic cells (DCs) and macrophages. This protein displays exceptional selectivity for GalNAc derivatives. The binding event is believed to trigger the antigen internalization/presentation, such as in the case of aberrant MUC1 overexpressed in several tumors histotype, but there are also strong evidence suggesting CLEC10A involvement in Ebola virus entry into DCs. The role of h-MGL in DCs function is not fully understood and therefore the development of potent inhibitors of h-MGL could be significant in gaining understanding of the purpose of this receptor in DCs biology and in improving DC-based vaccine development and immunotherapy. A compressive library with CLEC10A nM inhibitors and more than fifty MUC1 glycopeptides decorated with h-MGL ligands were synthesized. Their ability to be recognized and internalized by h-MGL and their ability to create immunological memory in a rabbit model is currently under evaluation. We were able to obtain the first crystal structure of CLEC10A and elucidate the GalNAc

binding mode with this protein, providing structural data long awaited in the glycobiology and immunology community.

Furthermore, we obtained data which could indicate involvement of hMGL-1 in HIV replication in DCs through a yet undefined mechanism.

References:

- [1]. Ernst B., Magnani J. L. (2009) Nat. Rev. Drug Discov. 8: 661-677.
- [2]. Van Vliet S. J., Saeland E., van Kooyk Y. (2008) Trends. Immunol. 29: 83-90.
- [3]. Eggink L. L., Roby K. F., Cote R., Hooper J. K. (2018) J. Immunother. Cancer 6:28.

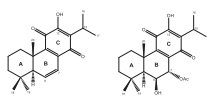
C.02 - Royleanones from *Plectranthus* spp. as P-glycoprotein inhibitors in multidrug resistant cellsVera M. S. Isca^{a,b}, Epole Ntungwe^a, Ricardo J. Ferreira^c, Tijana Stankovic^d, Amílcar Roberto^a, Milica Pesic^d, Daniel J. dos Santos^e, Carlos A.M. Afonso^b, Patrícia Rijo^{a,b}^aCenter for Research in Biosciences & Health Technologies (CBIOS), Universidade Lusófona de Humanidades e Tecnologias, 1749-024 Lisboa, Portugal^bInstituto de Investigação do Medicamento (iMed.ULisboa), Faculdade de Farmácia, Universidade de Lisboa, 1649-003 Lisboa, Portugal^cLaboratory of Molecular Biophysics, Uppsala University, Husargatan 3, 752 37 Uppsala, Sweden^dInstitute for Biological Research "Siniša Stanković", University of Belgrade, Despota Stefana 142, 11060 Belgrade, Serbia^eBioISI: Biosystems and Integrative Sciences Institute, Faculty of Sciences, University of Lisbon, Campo Grande, C8, 1749-016, Lisbon, Portugal**Abstract / Resumo da Comunicação**

Plectranthus genus is an important source of bioactive royleanones [1]. 6,7-Dehydroroyleanone (DHR) and 7 α -acetoxy-6 β -hydroxyroyleanone (AHR) are examples of lead molecules isolated in high amounts from *P. madagascariensis* and *P. grandidentatus*, respectively [2, 3]. Both diterpenes have hydroxyl groups suitable for derivatization, which have drawn attention to the possibility of exploring its reactivity, with the aim of improving the cytotoxicity of the lead compounds.

In this work, several hemi-synthetic reactions were performed with the intention of achieving a small library of compounds with enhanced cytotoxic potential, resorting to reactions of carbamoylation, benzylation or hydrogenation. The general toxicity of the synthesized products was evaluated through an *Artemia salina* model. The benzylation products of AHR had increased the toxicity of the original scaffold, which led us to believe that the benzylation is a key step for increasing the toxicity of this diterpene. A dibenzoylated derivative of AHR, previously synthesized, also demonstrated to enhance the cytotoxic properties [1]. Considering this, the synthesized compounds were also assayed by docking in a murine PgP structure [4] and promising results were obtained for the benzylation and dibenzoylation products, indicating that benzoyl residues are important groups for the bioactivity.

References:

- [1]. Ladeiras D. et al. (2016) Current Pharmaceutical Design. 22 (12): 1682-1714.
- [2]. Garcia C, et al. (2018) Future Med. Chem. 10(10): 1177-1189.
- [3]. Bernardes CES et al. (2018) Mol. Pharm. 15(4): 1412-1419.
- [4]. Ferreira RJ et al. (2012) J. Chem. Theory Comput. 8(6): 1853-1864

6,7-Dehydroroyleanone (DHR) and 7 α -acetoxy-6 β -hydroxyroyleanone (AHR) structures.

C.03 - Studies Towards the Total Synthesis of Tedanolide C

Lücke, Daniel, Kalesse, Markus

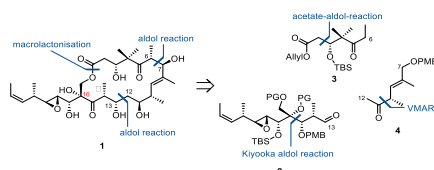
Institute for Organic Chemistry Leibniz University Hannover, Hannover, Germany

Abstract / Resumo da Comunicação

In 2005 Ireland and co-workers isolated the cytotoxic macrolide (IC₅₀ = 95.3 nM, HCT-116) tedanolide C from the marine sponge *Ircinia* sp.[1] The structure of the marine polyketide consists of an 18-membered macrolactone and a seven carbon atom long side chain. The molecule has twelve stereogenic centres in total, of which the tertiary alcohol at C16 is synthetically challenging. Until now, no successful total synthesis but five fragment syntheses have been published.[2],[3],[4],[5],[6] Furthermore, it needs to be mentioned that the absolute configuration of tedanolide C was determined by a combination of NMR experiments and molecular modeling.[1] Based on these experiments the proposed configuration shows some differences in the southwestern hemisphere of the molecule, compared to other members of the tedanolide family.[7],[8],[9] Considering this fact, we decided to aim for a diastereomeric tedanolide C, where the configuration of our target molecule 1 is similar to all other members of this natural product family. Our retrosynthetic approach divides the molecule into three fragments that should be connected via two aldol reactions followed by a macrolactonisation. All fragments have aldol-reactions as key steps which are a Kiyooka-aldol-reaction developed in our laboratories,[3] an acetate-aldol-reaction[10] and a vinylogous Mukaiyama-aldol-reaction (VMAR).[11]

References:

- [1]. Chevallier, C.; Bugni, T. S.; Feng, X.; Harper, M. K.; Orendt, A. M.; Ireland, C. M.; J. Org. Chem. 2006, 71, 2510-2513.
- [2]. Barth, R.; Roush, W. R.; Org. Lett. 2010, 12, 2342-2345.
- [3]. Bülow, L.; Naini, A.; Fohrer, J.; Kalesse, M.; Org. Lett. 2011, 13, 6038-6041.
- [4]. Smith, T. E.; Fink, S. J.; Levine, Z. G.; McClelland, K. A.; Daub, M. E.; Org. Lett. 2012, 14, 1452-1455.
- [5]. Geist, J. G.; Bart, R.; Roush, W. R.; Org. Lett. 2013, 15, 58-61.
- [6]. Zambrana, J.; Romea, P.; Urpi, F.; Org. Biomol. Chem. 2016, 14, 5219-5223.
- [7]. Schmitz, F. J.; Gunasekera, S. P.; Yalamanchili, G.; Hossain, M. B.; Van der Helm, D.; J. Am. Chem. Soc. 1984, 106, 7251-7252.
- [8]. Fusetani, N.; Sugawara, T.; Matsunaga, S.; Hirota, H.; J. Org. Chem. 1991, 56, 4971-4974.
- [9]. Meragelman, T. L.; Willis, R. H.; Woldemichael, G. M.; Heaton, A.; Murphy, P. T.; Snader, K. M.; Newman, D. J.; van Soest R.; Boyd, M. R.; Cardellina, J. H.; McKee, T. C.; J. Nat. Prod. 2007, 70, 1133-1138.
- [10]. Altmann; K.-H.; Bold, G.; Caravatti, G.; Denni, D.; Flörsheimer, A.; Schmidt, A.; Rihs, G.; Wartmann, M.; Helv. Chim. Acta 2002, 85, 4086-4110.
- [11]. Nagasawa, T.; Kuwahara, S.; Org. Lett. 2013, 15, 3002-3005.



C.04 - Multi-methodological characterization of *Cannabis sativa* L. inflorescences

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Abstract / Resumo da Comunicação

Cannabis sativa L. is an annual plant recently reintroduced in the European agronomical scenario, after decades of oblivion due to the association to the narcotic *Cannabis* (*Cannabis indica*) [1,2]. In fact, although the cultivation of *Cannabis sativa* L. was allowed, the Italian traditional varieties, appreciated all over the world, disappeared from the territory.

The present study [3], developed within the Regional Latium project (Protocol 85-2017-15069 CUP: B86C18000730002) titled Industrial Hemp: development and valorization of a sustainable new food chain, aimed at the characterization of *Cannabis sativa* L. selected varieties cultivated in well-defined areas of Lazio (an Italian region). Primary and secondary metabolites as well as principal cannabinoids important for the limits given by EEC Regulation and pharmaceutical aspects, were identified, quantified and monitored in three cultivars (Uso31, Felina and Ferimon) during four harvest time (June, July, August and September). A multi-methodological approach including untargeted (NMR) and targeted (HPLC, GC-MS) methodologies, was applied.

The obtained results will allow the selection of *Cannabis* varieties more suitable for the pedoclimatic conditions present in different areas of Lazio and for their applications in specific fields (e.g. food, pharmaceutical, cosmetic, fiber areas).

References:

- [1]. Regulation (EEC) No 1164/89 laying down detailed rules concerning the aid for fibre flax and hemp, Official Journal of the European Communities, 24-04-1989.
- [2]. Ministero delle Politiche Agricole e Forestali. Circolare 2 dicembre 1997, n. 734. Disposizioni relative alla coltivazione della *Cannabis sativa* L. (Canapa da "tiglio"), in G.U. n. 62 of 16-3-1998.
- [3]. Ingallina C et al., Inflorescences of *Cannabis sativa* L.: from chemical characterization to biological implications, in preparation.

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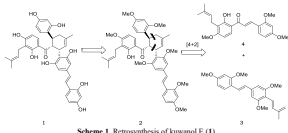
C.05 - Total synthesis of (±)-kuwanol E

Andrea Calcaterra, Valentina Iovine, Irene Benni, Rocchina Sabia, Ilaria D'Acquarica, Giancarlo Fabrizi, and Bruno Botta

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Abstract / Resumo da Comunicação

Kuwanol E (1) is a Diels-Alder adduct-type natural compound isolated from *Morus alba*¹, *Morus nigra*², and *Sorocea ilicifolia*³ plants. In a previous paper⁴ we showed as kuwanol E (1) is the most potent naturally occurring inhibitor of *Mycobacterium tuberculosis* (Mtb) protein tyrosine phosphatase B reported so far ($K_i = 1.6 \pm 0.1 \mu\text{M}$), a virulence factor secreted into the host cell by Mtb, and necessary for its survival. Compound 1, hence, is a good candidate as lead compound for the development of new antitubercular drugs. The availability of 1 from natural sources is very limited, a total synthesis will confirm the chemical structure and will provide enough amounts of the product for further biological investigations. Herein we reported the total synthesis of kuwanol E (1)⁵ via Diels-Alder reaction, a biomimetic intermolecular [4+2]-cycloaddition between stilbene-derived diene 3 and chalcone-derived dienophile 4. This key reaction permits the construction of a 6-membered monounsaturated ring, moreover, modulating the experimental conditions the regio- and stereochemical features of the products can be controlled.



The required Diels-Alder partners 3 and 4 have been synthesized in five and three steps, in 25% and 28% yield respectively. The cycloaddition between 3 and 4, promoted by Lewis acid catalysis, afforded the desired endo-kuwanol E heptamethyl ether (2), which has been deprotected to obtain kuwanol E (1) in 2% overall yield.

References:

- [1]. Hano Y, Nomura T, Ueda S. (1989). *Heterocycles* 29: 2035–2041.
- [2]. Ferrari F, Monacelli B, Messina I (1999). *Planta Med.* 65: 85–87.
- [3]. Ferrari F, Messina I (1995). *Phytochemistry* 38, 251–254.
- [4]. Mascarello A, Mori M, Chiaradia-Delatorre L D, Camila A, Menegatti O, Delle Monache F, Ferrari F, Yunes R A, Nunes R J, Terenzi H, Botta B, Botta M (2013). *PLoS One* 8: e77081.
- [5]. Iovine V, Benni I, Sabia R, D'Acquarica I, Fabrizi G, Botta B, Calcaterra A (2016). *J. Nat. Prod.* 79(10): 2495–2503

C.06 - Using Fluorescent Probes to optimize Tubulin-Binding Agents Research

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Abstract / Resumo da Comunicação

Tubulin and Microtubules have proved to be an outstanding target in cancer chemotherapy, being vinblastine, an antitumor agent targeting tubulin, the first anticancer magic bullet [1]. However, the search and evaluation of novel tubulin binders or derivatives is neither easy nor fast. Here we present a reliable and quick system (even useful for HTS) to study potential Tubulin-Binding Agents based on different fluorescent probes used in ligand competition assays [2-6]. These probes are designed for binding the well-characterized binding sites within tubulin and/or microtubules. Using them as tools in different methodology, this will broaden the tubulin research field.

References:

- [1]. Noble, R.L., C.T. Beer, and J.H. Cutts (1958). *Ann N Y Acad Sci.* 76 (3): p. 882-94.
- [2]. Diaz, J.F., et al., (2000). *J Biol Chem.* 275 (34): p. 26265-76.
- [3]. Menchon, G., et al., (2018). *Nat Commun.* 9 (1): p. 2106.
- [4]. Doodhi, H., et al., (2016). *Curr Biol.* 26 (13): p. 1713-21.
- [5]. Medrano, F.J., et al., (1991). *Biochemistry.* 30 (15): p. 3770-7.
- [6]. Canela, M.D., et al., (2014). *J Med Chem.* 57 (10): p. 3924-38.

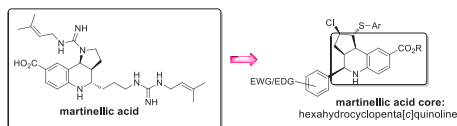
C.07 - New Allosteric Inhibitors of CDK2 and EGFR inspired by Martinellilic Acid

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Abstract / Resumo da Comunicação

Martinellilic acid is an alkaloid isolated from *Martinella iquitoensis* roots, that exhibits potent antagonist activity toward bradykinin (BK) B1 and B2 receptors. [1] Its biological activity, coupled with the unusual heterocyclic moiety, has made this alkaloid an attractive synthetic target.[2] In this field of interest, we synthesized a library of compounds inspired by martinellilic acid Scaffold. The activity of the obtained compounds was evaluated with various biological assays. [3]

References:

- [1]. Witherup, K. M.; Ransom, R. W.; Graham, A. C.; Bernard, A. M.; Salvatore, M. J.; Lumma, W. C.; Anderson, P. S.; Pitszenberger, S. M.; Varga, S. L. J. Am. Chem. Soc. 1995, 117, 6682
- [2]. Review: Nyerges, M. Heterocycles 2004, 63, 1685–1712
- [3] (a) M.S. Christodoulou, F. Caporuscio, V. Restelli, L. Carlino, G. Cannazza, E. Costanzi, C. Citti, L. Lo Presti, P. Pisani, R. Battistutta, M. Broggin, D. Passarella, G. Rastelli. ChemMedChem 2017, 12, 33-41; (b) L. Carlino, M.S. Christodoulou, V. Restelli, F. Caporuscio, F. Foschi, M. S. Semrau, E. Costanzi, A. Tinevella, L. Pinzi, L. Lo Presti, R. Battistutta, P. Storici, M. Broggin, D. Passarella, G. Rastelli. ChemMedChem 2018, 13, 2627–2634.

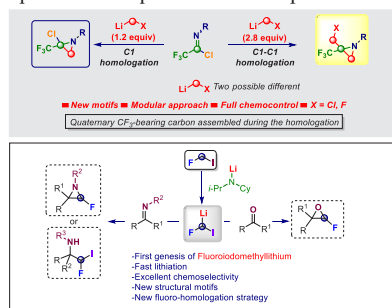
C.08 - Design and Developing New Chemical Reactions via Controlled Generation of Unstable Carbenoids Species

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Abstract / Resumo da Comunicação

The transfer of a reactive nucleophilic CH₂X unit into a preformed bond enables the introduction of the fragment featuring the exact and desired degree of functionalization through a single synthetic operation. The instability of metallated α -organometallic species often poses serious questions on the practicability of using this conceptually intuitive and simple approach for forming C-C or C-heteroatom bonds. The deep understanding of processes regulating the formation of these nucleophiles is a precious source of inspiration not only for successfully applying theoretically feasible transformations (i.e. determining how to employ a given reagent), but also for designing new reactions which ultimately lead to introduce molecular complexity via short experimental sequences as telescoped homologations or nucleophilic fluoromethylations.²



References

- (1) Castoldi, L.; Monticelli, S.; Senatore, R.; Ielo, L.; Pace, V. Chem. Commun. 2018, 54, 6692-6704.
- (2) (a) Pace, V.; Castoldi, L.; Mazzeo, E.; Rui, M.; Langer, T.; Holzer, W. Angew. Chem. Int. Ed. 2017, 56, 12677-12682. (b) Parisi, G.; Colella, M.; Monticelli, S.; Romanazzi, G.; Holzer, W.; Langer, T.; Degennaro, L.; Pace, V.; Luisi, R. J. Am. Chem. Soc. 2017, 139, 13648-13651. (c) Castoldi, L.; Pace, V. Nature Chem. 2018, 10, 1081-1082. (d) Ielo, L.; Touqeer, S.; Roller, A.; Langer, T.; Holzer, W.; Pace, V. Angew. Chem. Int. Ed. 2019, doi:10.1002/anie.201812525. (e) Monticelli, S.; Colella, M.; Pillari, V.; Tota, A.; Langer, T.; Holzer, W.; Degennaro, L.; Luisi, R.; Pace, V. Org. Lett. 2019, doi: 10.1021/acs.orglett.1028b04001.

C.09 - Improvement of antimicrobial and immunomodulatory properties by two L-to D-amino acid substitutions in the frog-skin peptide Esc(1-21)

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Abstract / Resumo da Comunicação

Antibiotic resistance is one of the most urgent threats to the public's health. Particularly, pulmonary infections by the bacterial pathogen *Pseudomonas aeruginosa* are the main cause of mortality especially in cystic fibrosis patients.[1] Naturally-occurring antimicrobial peptides (AMP) represent a new class of anti-infective agents that can limit the onset of resistance and that can be exploited for further biological/immunomodulatory properties. Recently, a strong antibacterial activity was found for the frog-skin derived peptide Esc(1-21). With the purpose to reduce its cytotoxicity and to increase its resistance to proteases, a diastereomer of Esc(1-21), containing two D-amino acids, i.e. Esc(1-21)-1c, was designed.[2] Compared to Esc(1-21), the diastereomer was: i) significantly less toxic towards mammalian cells, in agreement with its lower α -helical structure; ii) more effective against the biofilm form of *P. aeruginosa* and against *Pseudomonas* cells once internalized into bronchial cells; iv) more resistant to bacterial and human elastases. Moreover, the diastereomer was found (i) to have a higher activity than the all-L peptide in promoting migration of bronchial epithelial cells and presumably in favoring re-epithelialization of damaged lung tissue; (ii) to disaggregate and detoxify the bacterial lipopolysaccharide and to inhibit cyclooxygenase-2 synthesis, albeit less than the wild-type peptide.[2,3] Based on these interesting biological properties, further studies are conceivable to develop Esc(1-21)-1c as a new drug with multiple functions.

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References:

- [1]. Chen C, Mangoni ML, Di YP (2017). *Sci Rep* 7(1):8548.
- [2]. Di Grazia A, Cappiello F, Cohen H, Casciaro B, Luca V, Pini A, Di YP, Shai Y, Mangoni ML (2015). *Amino Acids* 47(12):2505-19.
- [3]. Cappiello F, Di Grazia A, Segev-Zarko LA, Scali S, Ferrera L, Galletta L, Pini A, Shai Y, Di YP, Mangoni ML (2016). *Antimicrob Agents Chemother* 60(12):7252-7262.

C.10 - Stereoselective Synthesis and Biological Evaluations of RvD1_{n-3} DPA

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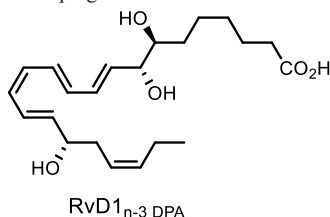
Abstract / Resumo da Comunicação

Specialized pro-resolving mediators (SPMs) display very potent anti-inflammatory and pro-resolving agonists effects towards G protein-coupled receptors (GPRs).1 The resolution of inflammation is now recognized to be an active process strictly regulated by individual families of SPMs and is therefore seen as a biomedical paradigm shift.2 SPMs are stereoselectively biosynthesized from ω -3 polyunsaturated fatty acids, such as docosahexaenoic acid (DHA) and docosapentaenoic acid (n-3 DPA), in the presence of lipoxygenases and cyclooxygenase-2.1

The first total synthesis of the specialized pro-resolving mediator RvD1n-3 DPA3 has been achieved using the underutilized sp3-sp3 Negishi cross coupling reaction and an alkyne hydrosilylation-protodesilylation protocol. The LC-MS/MS results of the synthetic material matched those of endogenously produced RvD1n-3 DPA allowing assignment of the absolute configuration of this lipid mediator. Biological evaluations revealed that this novel mediator displays low nanomolar pro-resolving properties in vivo and potently activates the GPR32 receptor. As such, this natural product is of interest as a lead compound towards developing novel immunoresolvents as putative new anti-inflammatory drugs.2,4.

References:

- [1]. Serhan C N, (2014). *Nature* 510: 92-101.
- [2]. Fullerton J N, Gilroy D W (2016). *Nat. Rev. Drug. Discov.* 15: 551-567.
- [3]. Tungen J E, Gerstmann L, Vik A, Matteis R D, Colas R A, Dalli J, Chiang N, Serhan C N, Kalesse M, Hansen T V, (2018), *Chem. Eur. J* 24: 1-6.
- [4]. (a) Vik A, Dalli J, Hansen T V, (2017). *Bioorg. Med. Chem. Lett.* 27: 2259-2266. (b) Serhan C N, (2017). *FASEB J.* 31: 1273-1288.



C.11 - Antitubercular activity and mitochondrial dysfunction of halimane and labdane diterpenoids from *Plectranthus ornatus* Codd.

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Abstract / Resumo da Comunicação

Medicinal plants from the *Plectranthus* genus are a valuable source of natural products such as diterpenes [1]. Mitochondrial dysfunctions (MD) have been associated with several pathologies such as ROS increase and uncontrolled *Mycobacterium tuberculosis* (Mtb) replication [2]. The electrochemical gradient produced by mitochondria generates the mitochondrial membrane potential (MMP), which is a key parameter for evaluating MD. Previous works have reported the cytotoxicity of *Plectranthus* diterpenoids and pointed their potential against *M. smegmatis* [3].

In this work, diterpenoids from *P. ornatus* Codd. (previously isolated) were evaluated for their cytotoxicity and for the mechanisms of cell death associated with MD in A549 cell line (human lung adenocarcinoma). One halimane HAL: (11R*,13E)-11-acetoxyhalima-5,13-dien-15-oic acid and labdane diterpenes PLEC: Plectromatine C and a (1:1) mixture MRC: 1,6-di-O-acetylforskolin:1,6-di-O-acetyl-9-deoxyforskolin were evaluated.

Our pioneer study showed that only HAL and PLEC were cytotoxic (IC₅₀=60 and 8 µg.mL⁻¹, respectively). Also, the ROS level observed after 1h was significantly higher ($p < 0.01$) with HAL and this effect was maintained for up to 48h. All compounds were able to decrease mtDNA copy number, but only HAL increased MMP and exhibited DNA damage of 8.78 lesions per 10 kb (ND5 region).

In conclusion, HAL has a cytotoxic effect associated with MD on lung cancer cells, that may be further evaluated on the Mtb replication mechanism. Additional studies are ongoing, aiming to unveil the coexistence of tuberculosis and lung cancer that has remained controversial, since the middle of the 19th century.

References:

- [1] Rijo, P., et al. *Magn Reson Chem* 2005, 43, 595–8;
- [2] Sakamuru, S., et al. *Methods Mol Biol*. 2016, 1473, 17–22;
- [3] Rijo, P., et al. *Biochem Syst Ecol*. 2007, 35, 215–21

C.12 - From PAINS natural products to preclinical candidates through computational studies

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Abstract / Resumo da Comunicação

Compounds that can interfere with bioassays via a number of different mechanisms are termed Pan Assay Interference compounds (PAINS). The concept of PAINS is also relevant for natural products, although the biological readout is an important factor that must be brought into consideration.(1)

In the search for novel drug candidates for the therapy of AIDS/HIV that could act on novel drug targets, we recently established a multidisciplinary screening funnel boosted by computational studies to discover non-covalent inhibitors of the HIV-1 nucleocapsid protein (NC). The NC is indeed considered as a promising antiretroviral target, particularly to overcome drug resistance to currently licensed anti-HIV drugs.(2-5)

Among multiple lead compounds identified, nordihydroguaiaretic acid (NDGA) emerged as a novel natural product inhibitor of NC. NDGA's mechanism of action was extensively characterized by using NMR, mass spectrometry, fluorescence spectroscopy and computational modeling.(6) NDGA was also shown to inhibit NC and the replication of wild-type and multiple drug-resistant HIV-1 strains in the low micromolar range with moderate cytotoxicity.(6) Notwithstanding, NDGA qualifies as a PAINS compound, and its development might be impaired by chemical reactivity. Thus, a virtual screening of the IRBM proprietary library for catechol-like pharmacophores led to identify the 5,6-dihydroxypyrimidine moiety as privileged scaffold of NC inhibitors. Subsequent hit-to-lead optimization driven by computational studies led to the development of an orally-bioavailable NC inhibitor with improved antiretroviral efficacy, which is currently under investigation in preclinical studies.

References:

- [1]. Baell, B.J. *J. Nat. Prod.*, 2016, 79 (3), pp 616–628
- [2]. Mori, M.; et al. *Curr Top Microbiol Immunol* 2015, 389, 53-92.
- [3]. Demene, H.; et al. *Biochemistry* 1994, 33, 11707-16.
- [4]. Mori, M.; et al. *J Chem Inf Model* 2011, 51, 446-54.
- [5]. Mori, M.; et al. *ACS Chem Biol* 2014, 9, 1950-5.
- [6]. Mori, M.; et al. *ACS Chem Biol* 2018, 13 (1), 253–266.

C.13 - *Plectranthus* spp. abietane diterpenoids as a new class of promising activators of PKCs

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Abstract / Resumo da Comunicação

Cancer is one of the main causes of death worldwide. Protein Kinase C (PKC) family has been the subject of intense research in the context of human cancer and is a promising therapeutic target in cancer [1]. Diterpenes are frequently found in the *Plectranthus* spp., and have a wide spectrum of biological activity, namely anticancer properties [2]. The diterpenoid 7 α -acetoxy-6 β -hydroxyroyleanone (AHR) obtained from *P. grandidentatus* exhibits low cytotoxicity and the basic requirements methods for the development of pharmaceutical formulations based on AHR as a lead, such as extraction optimization and structural and thermal properties characterization [3]. These features suggests that AHR can be used as an interesting lead for drug development. Using a previously developed yeast-based screening assay to search for modulators of PKC isoforms [4], a small library of abietane derivatives was tested for their ability to activate PKC isoforms from classical (α , α ; β , β), novel (δ , δ ; ϵ , ϵ) and atypical (ζ , ζ) subfamilies. The results obtained revealed potent activators of PKC family proteins, namely: a selective activator of PKC δ , the 7 α -acetoxy-6 β -benzoyloxy-12-O-benzoylroyleanone (Roy-Bz). The patented diterpene RoyBz was obtained using AHR as starting material. Roy-Bz potently inhibited the proliferation of colon cancer cells by inducing a PKC δ -dependent mitochondrial apoptotic pathway involving caspase-3 activation. In HCT116 colon cancer cells, the results indicate that Roy-Bz targets drug resistant cancer stem cells, preventing tumor dissemination and recurrence. Moreover, our findings support a tumor suppressive function of PKC δ in colon cancer. Altogether, these results indicated that promising activators of PKCs with high potency and isoform-selectivity may emerge from the exploitation of this new family of abietane diterpenoids [5].

References:

- [1]. Bessa C et al. (2018) Cell Death Dis. 9(2).
- [2]. Ladeiras D et al. (2016) Current Pharmaceutical Design. 22 (12): 1682-1714.
- [3]. Bernardes CES et al. (2018) Mol. Pharm. 15(4): 1412–1419.
- [4]. Coutinho et al., (2009) Biochem Pharmacol., 78:449-459.
- [5]. L. Saraiva et al., “Roy-Bz: a small molecule activator of Protein Kinase Cdelta (PKCdelta)” Patent Ref PCT/IB2017/050633.