Bridging structural biology and drug discovery - II

Report of Contributions

https://eventi.mlib.ic.cnr.it/e/35

Contribution ID: **1** Type: **not specified**

Discussioni e Conclusioni

Wednesday, 11 May 2022 12:30 (30 minutes)

Speakers: Dr.ssa Cinzia Giannini (CNR - Istituto di Cristallografia) e Prof. Francesco Leonetti (Dipartimento di Farmacia – Scienze del Farmaco, Università degli Studi di Bari)

Contribution ID: **2** Type: **not specified**

Introduzione e presentazione degli istituti

Wednesday, 11 May 2022 09:30 (10 minutes)

Presenters: Dr GIANNINI, Cinzia (CNR - Istituto di Cristallografia); Prof. LEONETTI, Francesco (Dipartimento di Farmacia – Scienze del Farmaco, Università degli Studi di Bari)

Session Classification: Sessione

Contribution ID: **3** Type: **not specified**

Design of mucoadhesive drug delivery systems

Wednesday, 11 May 2022 09:40 (20 minutes)

The mucosal barrier is present at the level of several organs and represents the pathway that limits drug absorption at the level of the underlying epithelial membranes. Many polymers, both natural and synthetic, are known to exhibit mucoadhesive properties and recently it has also been shown that the presence of free thiol groups in the polymer backbone further increases mucoadhesion. (Perrone et al., 2018). The increased capacity of thiolate polymers, called thiomers, is due to the formation of disulfide bonds with the cysteine-rich mucin subdomains of the mucus lining the mucosa. This allows for more pronounced adhesion of the polymer to biological surfaces. Thus, the use of a suitable drug delivery system based on the use of thiolated mucoadhesive polymers can improve the bioavailability of drugs through the mucosal route. Indeed, by increasing the effectiveness of adhesion, and thus the residence time of this drug delivery system on the mucosal layer, the drug concentration at the site of absorption is correspondingly increased. The functionality and efficiency of thiomers can be significantly reduced by the oxidation phenomena of thiol groups, which can influence the interaction with cysteine rich subdomains present on the mucosa, thus limiting their mucoadhesive characteristics. To prevent this phenomenon, S-protected thiomers, characterized by the presence of pyridilic disulphides, have been studied. S-protected thiomers present the advantage that they do not cross-link during storage even in aqueous solution, so they increase the stability of thiols over a large pH range. Moreover, pre-activated thiomers are more reactive than simple thiolated polymers. In a recent study Racaniello et al. used 2-mercaptonicotinic acid, in the dimeric form, to protect thiol groups of thiolated cyclodextrin and obtain a novel nasal mucoadhesive drug delivery system of corticosteroid drug Budesonide. Moreover, it is reported in the literature that the combination of thiol groups and nanoparticulate (NPs) systems offers promising permeation enhancing effects in relation to mucoadhesive and mucus diffusion features, reason why many of the thiolated polymers are used for the formulation of NPs drug delivery systems. Preparation of NPs starting with thiolated polymers include different methods such as nanoprecipitation, spray drying, self-assembly and emulsification techniques (Hock et al., 2021). Once obtained, the thiomer based drug delivery systems must be tested for their mucoadhesive properties. A number of methods have been outlined; mostly commonly used in vitro methods include the use of a rotating cylinder method, rheology, tensile strength tests and flow-through tests.

1) Eur J Pharm Biopharm. 2018 Nov;132:103-111.
2) Int J Pharm. 2021 Jun 15;603:120728.
3) Adv Sci (Weinh). 2022 Jan;9(1):e2102451.

Presenter: Prof. LAQUINTANA, Valentino (Dipartimento di Farmacia – Scienze del Farmaco, Università degli Studi di Bari)

Session Classification: Sessione

Contribution ID: **7** Type: **not specified**

Facing sustainable challenges in the FLAME lab: an overview on main synthetic tactics using flow technology to access new chemical space

Wednesday, 11 May 2022 11:30 (20 minutes)

Microreactor technology and flow chemistry could play an important role in the development of green and sustainable synthetic processes.1 In this presentation, some recent relevant examples in the field of flash chemistry, catalysis, hazardous chemistry and continuous flow processing of metallated species are described.2 Selected examples highlight the role that flow chemistry could play in the near future for a sustainable development. In addition, this overview focusses on new synthetic tactics for the straightforward preparation of hardly accessible structural motifs and functional groups of sulfur (IV) and (VI) as sulfilimines, sulfinimidates, sulfinamidines and sulfinamide esters, by using simple metal-free protocols in bench and flow conditions. 3 In situ FT-IR investigations and quantum mechanical/nuclear magnetic resonance (NMR) approaches are widely used for the mechanistic hypothesis of studied reactions as well as for structural and configurational assignment of isolated organic compounds generally supported also by crystallographic analysis.

1) Fanelli, F., Parisi, G., Degennaro, L., Luisi, R. Contribution of microreactor technology and flow chemistry to the development of green and sustainable synthesis (2017) Beilstein Journal of Organic Chemistry, 13, pp. 520-542.
2) a) Musci, P., von Keutz, T., Belaj, F., Degennaro, L., Cantillo, D., Kappe, C.O., Luisi, R. Flow Technology for Telescoped Generation, Lithiation and Electrophilic (C3) Functionalization of Highly Strained 1-Azabicyclo[1.1.0]butanes (2021) Angewandte Chemie - International Edition, 60 (12), pp. 6395-6399. b) Colella, M., Musci, P., Cannillo, D., Spennacchio, M., Aramini, A., Degennaro, L., Luisi, R. Development of a Continuous Flow Synthesis of 2-Substituted Azetines and 3-Substituted Azetidines by Using a Common Synthetic Precursor (2021) Journal of Organic Chemistry, 86 (20), pp. 13943-13954. c) Colella, M., Tota, A., Takahashi, Y., Higuma, R., Ishikawa, S., Degennaro, L., Luisi, R., Nagaki, A. Fluoro-Substituted Methyllithium Chemistry: External Quenching Method Using Flow Microreactors (2020) Angewandte Chemie - International Edition, 59 (27), pp. 10924-10928. d) Monticelli, S., Colella, M., Pillari, V., Tota, A., Langer, T., Holzer, W., Degennaro, L., Luisi, R., Pace, V. Modular and Chemoselective Strategy for the Direct Access to α-Fluoroepoxides and Aziridines via the Addition of Fluoroiodomethyllithium to Carbonyl-Like Compounds (2019) Organic Letters, 21 (2), pp. 584-588.
3) a) Zenzola, M., Doran, R., Degennaro, L., Luisi, R., Bull, J.A. Transfer of Electrophilic NH Using Convenient Sources of Ammonia: Direct Synthesis of NH Sulfoximines from Sulfoxides (2016) Angewandte Chemie - International Edition, 55 (25), pp. 7203-7207. b) Andresini, M., Tota, A., Degennaro, L., Bull, J.A., Luisi, R. Synthesis and Transformations of NH-Sulfoximines (2021) Chemistry - A European Journal, 27 (69), pp. 17293-17321. c) Andresini, M., Spennacchio, M., Colella, M., Losito, G., Aramini, A., Degennaro, L., Luisi, R. Sulfinimidate Esters as an Electrophilic Sulfinimidoyl Motif Source: Synthesis of N-Protected Sulfilimines from Grignard Reagents (2021) Organic Letters, 23 (17), pp. 6850-6854. d) Andresini, M., Colella, M., Degennaro, L., Luisi, R. Hypervalent iodine (III) reagents and ammonia as useful combination for highly chemoselective N-transfer to low-valent organosulfur compounds and amines (2021) Arkivoc, 2022 (4).

Presenter: Prof. DEGENNARO, Leonardo (Dipartimento di Farmacia – Scienze del Farmaco, Univer-

sità degli Studi di Bari)

Session Classification: Sessione

Contribution ID: **8** Type: **not specified**

Unravelling the Primary Structural Determinants Essential for Proneurotrophins Biological Functions by a Combined Evolutionary and Structural Approach

Wednesday, 11 May 2022 10:00 (20 minutes)

Nerve Growth Factor, Brain-Derived Neurotrophic Factor, Neurotrophin 3 and Neurotrophin 4 are known to play a range of crucial functions in the developing and adult peripheral and central nervous systems. Initially synthesized as precursors that are cleaved to release C-terminal mature forms, they act through two types of receptors, the specific Trk receptors and the pan-neurotrophin receptor p75NTR, to initiate survival and differentiated responses. Recently all the proneurotrophins but proNT4 have been demonstrated to be not just inactive precursors, but signalling ligands that mediate opposing actions in fundamental aspects of the nervous system with respect to the mature counterparts through dual receptor complexes formation with sortilin, a member of the VPS10 family, and p75NTR. Despite the functional relevance, the molecular determinants underpinning the interactions between the pro-domains and their receptors are still elusive probably due to their intrinsically disordered nature. Here we present an evolutionary approach coupled to an experimental study aiming to uncover the structural and dynamical basis of the biological function displayed by proNGF, proBDNF and proNT3 but missing in proNT4. A bioinformatic analysis allowed elucidating the functional adaptability of the proNTs family in vertebrates, identifying conserved key structural features. The combined biochemical and SAXS experiments shed lights on the structure and dynamic behaviour of the human proNTs in solution, giving insights on the evolutionary conserved structural motifs, essential for the multifaceted roles of proNTs in physiological as well as in pathological contexts (1).

1) Comput. Struct. Biotechnol. J. (2021) 19:2891-2904. doi: 10.1016/j.csbj.2021.05.007.

Presenter: Dr COVACEUSZACH, Sonia (CNR - Istituto di Cristallografia)

Session Classification: Sessione

Contribution ID: **9** Type: **not specified**

A reliable cellular model-based platform for pharmacological preclinical studies on Tubular Aggregate Myopathy and SOCE-related muscle disorders

Wednesday, 11 May 2022 12:10 (20 minutes)

Tubular aggregates myopathy (TAM) is a hereditary ultra-rare muscle disorder, actually without a cure, characterized by progressive weakness, myalgia or myastenic features. Biopsies from patients with TAM show the presence of tubular aggregates (TAs) originated from sarcoplasmic reticulum (SR) (1). TAs formation is triggered by functional consequences due to disruption in the SR-T-tubule junction related to altered Ca2+ homeostasis. Indeed, TAM is caused by gain-of-function mutations in STIM1 or ORAI1, proteins responsible for Store-Operated-Calcium-Entry (SOCE), a pivotal mechanism in cellular calcium signaling and in maintaining cellular Ca2+ balance (2). The mechanisms underlying muscle weakness and TAs formation from altered Ca2+ homeostasis in skeletal muscle of affected individuals remain to be clarified. To date, most of the STIM1 and ORAI1 mutations has been functionally studied in heterologous expression systems with consequent limitations in terms of disease model reliability and translatability of drug efficacy in humans (2). Although the current availability of some animal models get a hopefully chance in this context (3,4), murine models only partially replicate muscle symptoms observed in TAM patients. In our laboratory we recently created, for the first time, a reliable cellular model usefull for TAM preclinical studies, consisting of myoblasts and myotubes deriving from TAM patients' biopsy carrying Leu96Val STIM1 mutation. By using a plethora of techniques ranging from cytofluorimetry and high content imaging to molecular biology, we demonstrated that STIM1 mutation causes an increase of resting Ca2+ concentration associated with an augmented SOCE activation and proved that differentiating Leu96Val STIM1 myoblasts persisted in a mononuclear state, resulting in a reduced number of multinucleated myotubes with distinct morphology and different geometry of mitochondrial network. Our study provides novel evidences about the correlation between SOCE activation, mitochondrial sufferance and defecting myogenesis in TAM finally highlighting that STIM1/ORAI1 proteins can be considered promising therapeutic targets (5). Importantly, SOCE dysfunction is also observed in various skeletal muscle wasting disorders such as muscular dystrophy, sarcopenia and cachexia (6). Thus, our future purpose will be to use the preclinical pharmacological platform settled in our laboratories for characterizing patient- derived cellular models in order to define related-disease endpoints and to test patient-specific drugs. As it is usually desired for neuromusculuar disorders (7,8), a such experimental approach could finally allow a reliable translation in the clinical management of SOCE-related muscular disorders.

1) Böhm J. et al., Acta Neuropathol. 2018
2) Morin G. et al., Hum Mutat 2020
3) Cordero-Sanchez C. et al., Dis. Model Mech.2020
4) Silva-Rojas, R. et al., Hum. Mol. Genet. 2019
5) Conte E et al., Front Cell Dev Biol. 2021
6) Conte E et al., Cells. 2021
7) Silva-Rojas R. et al., Front Physiol. 2020
8) Van Putten M. et al., Dis. Model. Mech. 2020

Presenter: Dr CONTE, Elena (Dipartimento di Farmacia – Scienze del Farmaco, Università degli Studi di Bari)

Session Classification: Sessione

Contribution ID: **11** Type: **not specified**

Single Crystal X-Ray Crystallography lights up pharmaceutical compounds: main investigations and structural insights

Wednesday, 11 May 2022 11:10 (20 minutes)

X-ray Crystallography plays a crucial role in the structural investigation of drugs and provides a fundamental support for drug design and quality control in the drug development process [e.g., monitoring and quality check of Active Pharmaceutical Ingredient (API) and finished products]. Depending on the size of crystals (i.e., fraction of mm or a few μm), the kind of source (i.e., X-ray laboratory source or synchrotron radiation), or the wanted structural information, the crystallographic study can be carried out by single crystal or microcrystalline powder diffraction data. X-ray powder diffraction data (XRPD) provide a bulk information and allow to perform qualitative and quantitative phase analysis, detection of impurity phase(s), study of phase transitions, estimation of the crystallinity degree and percentage of the amorphous component, investigation of polymorphism, drug quality control and crystal structure solution [1,2]. In case of laboratory X-ray sources, if crystals of suitable size (i.e., fractions of mm) are available, their structural characterization can be carried out by single crystal X-ray diffraction data (SCXRD), via structure determination process, to be preferred to the structure solution by XRPD, because, thanks to the best quality of SCXRD, a more detailed structure model can be obtained and more complex structures can be successfully characterized. Contrarily to the case of XRPD, SCXRD allow to locate H atoms via difference Fourier synthesis, and, consequently, to accurately detect the main interatomic interactions. The correct position of H atoms enables also to establish whether a material exists as a salt or cocrystal phase [3]. A correct and accurate crystal structure determination is fundamental to confirm the expected crystal structure or reveal a new one, e.g., a new polymorph, that can have different physical properties (e.g., in terms of solubility, bioavailability,…) [3,4]; determination of the absolute configuration, that can be a critical step for the pharmaceutical industry: opposite enantiomers of a drug can have different biological properties [5]. Nowadays, the structure solution process by SCXRD is usually a routinary task and, at the same time, is greatly useful for understanding the structure-property relationship of drugs and providing key elucidating answers both to pharmaceutical sciences and industries. The increasing access to non-conventional powerful X-ray sources (i.e., synchrotron radiation) allows to reduce the size of the single crystals that can be successfully investigated and to enhance the complexity of the crystal structures that can be solved by SCXRD. Examples of application of single crystal diffraction to investigate pharmaceutical compounds will be shown, together with some cases of structure solution based on single-crystal synchrotron X-ray microdiffraction data, that allowed to characterize microcrystals for which, due to their small size, the use of synchrotron radiation revealed itself an obliged choice, i.e., the only way for succeeding in solving the crystal structure [6,7].

[1] Powder Diffraction Theory and Practice, Eds. R.E. Dinnebier and S.J.L. Billinge, (2008). RSCPublishing, Cambridge, UK.
[2] A. Altomare, C. Cuocci, G.D. Gatta, A. Moliterni and R. Rizzi (2017). 'Methods of Crystallography: powder X-ray diffraction', EMU Notes in Mineralogy, Vol. 19, Chapter 2, 79-138.
[3] Special Issue on Pharmaceuticals, drug discovery and natural products (2013). Acta Cryst C. 69, Ed. C. S. Frampton.
[4] Virtual Issue on Polymorphism (2011). Acta Cryst C., Ed. A. Linden, https://journals.iucr.org/special_issues/2011/polymo
[5] Virtual Issue on Absolute structure (2012). Acta Cryst C., Ed. H. D. Flack https://journals.iucr.org/special_issues/2012/abs
[6] Giacobbe, C., Di Giuseppe, D., Zoboli, A., Lassinantti Gualtieri, M., Bonasoni, P., Moliterni, A., Corriero, N., Altomare, A., Wright, J., Gualtieri, A.F. (2021). IUCrJ 8, 76-86. DOI:10.1107/S2052252520015079
[7] Titi, A., Touzani, R., Moliterni, A., Giacobbe, C., Baldassarre, F., Taleb, M., Al-Zaqri, N., Warad,

I. ‘Ultrasonic clusterization process to prepare [(NNCO)6Co4Cl2] as novel double-open-Co4O6 cubane cluster: SXRD-interactions, , physicochemical, thermal, and Bio-mimicking of catecholase activity’, (2022). ACS Omega, Submitted.

Presenter: Dr MOLITERNI, Anna (CNR - Istituto di Cristallografia)

Session Classification: Sessione

Contribution ID: **13** Type: **not specified**

Natural and synthetic effectors of alpha-synuclein: features and interplays

Wednesday, 11 May 2022 11:50 (20 minutes)

Parkinson's disease (PD) and α-synucleinopathies are characterized by the progressive loss of neuronal cells and the decline of cognitive and motor functions. Oxidative stress, dyshomeostasis of metal ions and α-synuclein (αSyn) should be key factors in the development of these disorders1,2. Moreover, the aggregation of αSyn is a crucial event in the pathogenesis of α-synucleinopathies. Metal-protein interactions play an important role in αSyn aggregation and might represent a link between the pathological processes of protein aggregation, oxidative damage, and neural death. High Copper concentration is detected the cerebrospinal fluid of PD patients, as well as in the Lewy bodies, the intracellular aggregates of αSyn. Moreover, Copper regulates αSyn intracellular localization and cytotoxicity2. Lipoxidation and carbonylation have also been observed in neurodegenerative diseases. αSyn seems to induce lipid peroxidation and, conversely, αSyn carbonylation has been found in PD. In particular, acrolein (ACR) and 4-hydroxy-nonenal (HNE) have been reported to affect the aggregation process of αSyn3. The interplay between ACR, copper, and αSyn has been recently investigated4. Moreover, we comprehensively assessed the interaction with αSyn ability and inhibitory properties in preventing α-Syn aggregation of a series of glyco- and dipeptide-conjugates of 8-hydroxyquinoline, well-known molecules that provide neuroprotection in neurodegenerative disorders.

1) Jomova K, Vondrakova D, Lawson M, Valko M, Mol. Cell. Biochem. 2010, 345, 91-104.
2) Binolfi A, Quintanar L, Bertoncini CW, Griesinger C, Fernández CO, Coord. Chem. Rev. 2012, 256, 2188-2201.
3) Wang YT, Lin HC, Zhao WZ, Huang HJ, Lo YL, Wang HT, Lin AMY, Sci. Rep. 2017, 7, 45741.
4) Falcone, E., Ahmed, I.M.M., Oliveri, V., Bellia, F., Vileno, B., El Khoury, Y., Hellwig, P., Faller, P., Vecchio, G. Chem. Eur. J. 2020, 26, 1871.

Presenter: Dr BELLIA, Francesco (CNR-Istituto di Cristallografia)

Session Classification: Sessione

Contribution ID: **14** Type: **not specified**

Targeting the undruggable: preliminary studies on a promising beta-catenin inhibitor

Wednesday, 11 May 2022 10:40 (20 minutes)

The WNT signal transduction cascade is a key regulatory pathway during embryonic development and adult tissue homeostasis. In the canonical WNT pathway, the armadillo repeat protein β-catenin serves as the central signaling molecule by engaging in crucial protein-protein interactions (PPIs) with both positive and negative effectors of the pathway and by linking WNT ligand mediated pathway activation to target gene induction in the nucleus. Aberrant WNT pathway activation, leading to nuclear accumulation of β-catenin, is considered as a key driver event for initiation and progression of many cancers, including colorectal and hepatocellular carcinomas. Despite significant efforts made to identify modulators or inhibitors of WNT pathway, β-catenin is still deemed by most as an "undruggable" target and specific drugs against the signaling pathway for clinical treatment have not been approved yet. Here we present our preliminary data describing a promising interaction between the β-catenin armadillo repeats and the non-peptidic small molecule RS6452. In particular, results obtained by Surface Plasmon Resonance direct binding assay along with the structural investigation of the protein/ligand complex by X-ray crystallography will be presented, highlighting the scientific relevance of this discovery and focusing on the further experimental strategy to assess the potential of RS6452 as new anticancer drug.

Presenter: Dr CAPELLI, Davide (CNR - Istituto di Cristallografia)

Session Classification: Sessione

Contribution ID: **15** Type: **not specified**

Nutraceutics and Functional Foods: work in progress

Wednesday, 11 May 2022 10:20 (20 minutes)

Over the years, consumers' awareness of the close relationship between food and health has increased. On the basis of this strong cultural drive, the attention towards the health and nutraceutical aspects of food has grown, thus becoming one of the most important criteria influencing consumers' purchasing choices and generating new market opportunities. Nutraceutics, an innovative branch of pharmaceutics, is aimed at this sector and in Italy it has increased to such an extent that it has become one of the European countries most attentive to these products. This new frontier can be an important driving force for socio-economic growth, both in terms of turnover and new professions. The research group has experience in the field of Food Chemistry and Food Technologies applied to the field of Nutraceutics, Functional Foods and Food Technologies as well as in the field of Pharmaceutical Chemistry. It has expertise in design, prototyping, characterization, property protection and scientific communication of new ingredients, products and processes. The ongoing regional, national and European projects will be illustrated, which have intercepted food chains with high added value in terms of raw materials, candidate as functional foods or sources of nutraceuticals, of the Mediterranean basin such as extra virgin olive oil, wine, microgreens, cherries, carob and new productions such as the Giant Bamboo (Pyllostachis Pubescens). The multidisciplinary nature of the sector imposes the need to "look" at the food from different points of view that concern analytical-extractive, technological-formulative-engineering, nutrigenomic, as well as economic-legislative and communication skills. To this end, the Multidisciplinary Laboratory of Functional Food Chemistry and Technology uses internal human resources but also many collaborations at national and international level, with the aim of producing a "Farm to Fork" research useful to the health and medical sector and with a high TRL. The research team is specialized in the development and scale-up of processes based on emerging technologies (microwave, ultrasound) for the production of different types of molecules of industrial interest for the pharmaceutical, cosmetic, nutraceutical, food and green chemistry sectors. Finally, a special focus is given to emerging disciplines such as Precision Cooking and FOP (front of packages) labeling, links between nutraceutics and precision nutrition, which will facilitate in the near future the precise determination of the diet for each individual, in relation to its biological, metabolic and genetic parameters.

Presenter: Prof. CORBO, Filomena (Dipartimento di Farmacia – Scienze del Farmaco, Università degli Studi di Bari)

Session Classification: Sessione