DESENVOLVIMENTO, AVALIAÇÃO E QUANTIFICAÇÃO DE

BIOMOLÉCULAS

Autores: Dulce Teixeira[1], Ricardo Ferraz[1,2], Cristina Prudêncio[1,3], Mónica Vieira[1,3]

[1]Ciências Químicas e das Biomoléculas/CISA, Escola Superior de Saúde—Instituto Politécnico do Porto, Porto, Portugal), [2]LAQV-REQUIMTE, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto,[3]i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal

Abstract

The synthesis of new biomolecules, the evaluation of their potential biological activity or therapeutic application, as well as the monitoring of intervening biomolecules in pathological contexts is of great interest to the scientific, medical and pharmaceutical community[1,2,3].

In the validation of synthesis, extraction and biological interaction methods, chromatographic methods play a fundamental role in the identification and quantification of these biomolecules of interest, whether these are potential new drugs, biomarkers or environmental indicators[4].

In order to contribute to the identification and quantification of biomolecules with potential (bio) technological application, new chromatographic methodologies have been developed, namely based on liquid chromatography, using HPLC-DAD[5,6].

In the development of new biomolecules, the bioactivity of compounds with potential application has been studied, such as Quinoxaline derivatives5, 6, 7. In addition to the antimicrobiological action, the effect of this family of compounds in the modeling of oxidative stress in pathological context has been evaluated [7].

The synthesis of new biomolecules, the evaluation of their potential biological activity or therapeutic application, as well as the monitoring of intervening biomolecules in pathological contexts is of great interest to the scientific, medical and pharmaceutical community[1,2,3].

The study of quinoxaline and its derivatives has become a subject of interest in recent years due to their wide variety of biological activities as well as therapeutic applications. Since they are rare in nature, synthetic quinoxalines are included in various antibiotics such as echinomycin, levomycin and actinomycin, well-known to inhibit the growth of Gram-positive bacteria and are also active against transplant tumors[3].

In search of alternatives to antimicrobial agents currently in use, and in order to respond to the landscape of loss of efficacy due to the emergence of resistance, several research groups are evaluating several families of compounds, among them the quinoxaline derivatives family, which presented several other potential biological applications[3,4].

Modifying quinoxaline's structure is possible to obtain a wide variety of compounds with different biological properties.

In the last two decades, several quinoxaline derivatives have been tested and presented antimicrobial activity, as antifungal and antibacterial agents. The antibacterial activity observed covers both Gram-negative and Gram-positive bacteria, including Mycobacterium species. There are also data pointing to activity against multidrug resistant Mycobacterium tuberculosis. Several studies have been described, concerning synthesis and biological activity of

a large amount of quinoxalines. Some quinoxaline 1,4-di-N-oxide derivatives have been shown to inhibit M. tuberculosis to a rate of 99 to 100% . Antifungal activity for quinoxaline derivatives has been tested against Candida albicans. Researchers also reported quinoxaline derivatives high antifungal activity. There are quinoxaline derivatives that present anti-protozoan activity, especially anti-amoebic, against Plasmodium and Leishmania species.

Quinoxaline derivatives present other biological properties. Anticancer activity has been already studied, and results obtained are very promising. Recently, our research group has demonstrated anti-proliferative activity in several cancer cell lines.

Some of the compounds studied by our group presented potential as new drugs for antimicrobial activity chemotherapy since the MIC's determined present low values and cellular viability tests show the complete elimination of the bacterial strain. In addition, the cellular viability tests for an eukaryotic model, indicate low toxicity for the compounds tested[5].

In the validation of synthesis, extraction and biological interaction methods, chromatographic methods play a fundamental role in the identification and quantification of these biomolecules of interest, whether these are potential new drugs, biomarkers or environmental indicators6. For this purpose, our group has identified, developed and validated new analytical chromatographic methods, based on HPLC-DAD chromatography[6,7].

Molecules modified by interactions with reactive oxygen species (ROS) in the microenvironment, and those changed in response to increased redox stress, are considered biomarkers of oxidative stress. The nitration of tyrosine (Tyr) residues in proteins is associated with nitrosative stress. L-Tyr and protein associated Tyr are the target of various reactive-nitrogen species (RNS), resulting in the formation of free 3-nitrotyrosine (3-NT) and protein-associated 3-NT. It forms after the substitution of a hydrogen by a nitro group (NO_2) in the ortho position of the phenolic ring of the Tyr residues. Studies have suggest that 3-NT is likely to have a deleterious effect on protein function and less likely to be important in normal cellular function. The nitration of proteins is a common process that occurs under physiological conditions and the concentration of 3-NT in plasma of healthy humans is on the threshold of the nM-to-pM range.A significant increase in the extent of this process results in increased 3-NT levels in biological samples and has been associated with a wide range of diseases. A wide range of methods for 3-NT detection and quantification were developed during the last years, all of them presenting positive and negative features. Our investigation group developed a new method that exhibited a good specificity,

with no interference observed with 3-NT structural relatives, namely Tyr. This method revealed good precision and accuracy. Additionally, the same method, with detection at 356 nm, also allowed the successful detection and quantification of 3-NT in a wide variety of biological matrices. Therefore, and unlike other developed methods for 3-NT quantification, our HPLC-based method was successfully applied to a wide range of biological matrices, exhibiting a great performance in all of them and allowing the effective quantification of 3-NT[7].

The modeling of oxidative stress in pathological context has been proven to be very relevant. In order to apply our new method to different pathologies, the evaluation of oxidative stress in a tumoral environment was performed, combined with the potential antitumour activity of the quinoxaline derivatives studied, has well as their ability to promote radiation cellular sensitivity[8]. In this study, we investigated the oxidative status of quinoxaline-1,4-dioxides derivatives in modulating melanoma and glioma cell lines, based on previous results from the research group and their capability to promote cell damage by the production of ROS. Overall, the results obtained emphasized the influence of quinoxaline-1,4-dioxides derivatives on the in vitro modulation of oxidative stress in malignant melanocytes and brain tumor cell lines. Quinoxaline-1,4-dioxides derivatives sensitize radiation action, decrease antioxidant cell defenses, specifically glutathione synthesis and modify the intrinsic radio resistance performance of cell lines. However, more in-depth studies for these quinoxaline derivatives are required to undoubtedly confirm and support these preliminary results and their influence in these major pathways to understand the potential radiation chemo sensitizing action of quinoxaline derivatives[8].

In synthesis, the development of new potential drugs, as well as the validation of their biological activity may be an important tool to fight growing and threatening diseases. Also, the development of analytical methodologies, namely those based on chromatographic techniques as HPLC-DAD, help investigators to validate biological mechanisms, evaluate diseases' environments and elucidate potential new targets. In this sense, we try to help to meet this goal with our contribution.

Bibliografia e Artigos da autoria do(s) autor(es) que apoiam a linha de investigação

[1]M.D.M.C. Ribeiro da Silva, V.L.S. Freitas, M.A.A. Vieira, M.J. Sottomayor, W.E. Acree (2012) Energetic and structural properties of 4-nitro-2,1,3-benzothiadiazole, The Journal of Chemical Thermodynamics, 49, 146-153. doi: 10.1016/j.jct.2012.01.018.

[2] Maria D.M.C. Ribeiro da Silva, Mónica A.A. Vieira, Chelsea Givens, Stephanie Keown, William E. Acree Jr. (2006) Experimental thermochemical study of two polymethylpyrazine N,N-dioxide Derivatives, Thermochimica ata, 450, 67-70. doi: :10.1016/j.tca.2006.07.021.

[3] Joana A. Pereira a, Ana M. Pessoa, M. Natália D.S. Cordeiro, Rúben Fernandes, Cristina Prudêncio, João Paulo Noronha, Mónica Vieira (2015) Quinoxaline, its derivatives and applications: A State of the Art Review, European Journal of Medicinal Chemistry, 97, 664-672. doi: 10.1016/j.ejmech.2014.06.058.

[4] Mónica Vieira, Ricardo Ferraz, Rúben Fernandes, Cristina Prudêncio (2014) New Quinoxalines with Biological Applications, Biochemistry & Pharmacology, Open Access, 03(01). doi: 10.4172/2167-0501.1000e152.

[5] Mónica Vieira, Cátia Pinheiro, Rúben Fernandes, João Paulo Noronha, Cristina Prudêncio (2014) Antimicrobial activity of quinoxaline 1,4-dioxide with 2- and3-substituted Derivatives, Microbiological Research, 169, 287-293. doi: 10.1016/j.micres.2013.06.015.

[6] Dulce Teixeira, Rúben Fernandes, Cristina Prudêncio, Mónica Vieira (2016) 3-Nitrotyrosine quantification methods: Current concepts and future challenges, Biochimie, 125, 1-11. doi: 10.1016/j.biochi.2016.02.011.

[7] Dulce Teixeira, Cristina Prudêncio, Mónica Vieira (2017) Development of a new HPLC-based method for 3-nitrotyrosine quantification in different biological matrices, Journal of Chromatography B, 1046, 48-57. Doi: 10.1016/j.jchromb.2017.01.035.

[8] Silva L, Coelho P, Teixeira D, Monteiro A, Pinto G, Soares R, Prudêncio C, Vieira M. (2020) Oxidative Stress Modulation and Radiosensitizing Effect of Quinoxaline-1,4-Dioxides Derivatives. Anticancer Agents Med Chem. 20(1):111-120. doi: 10.2174/1871520619666191028091547.