Dutra et al., Biochem Pharmacol (Los Angel) 2016, 5:3
DOI: 10.4172/2167-0501.1000e183

Biochemistry & Pharmacology: Open Access

Editorial Open Access

# The Paradigma of the Interference in Assays for Natural Products

**Luiz Antonio Dutra, Thais Regina Ferreira de Melo, Rafael Consolin Chelucci, Chung Man Chin and Jean Leandro dos Santos***

*Faculty of Pharmaceutical Sciences, UNESP-The Universidade Estadual Paulista, Araraquara, SP, Brazil*

Interference in assays (IA) is a common phenomenon and a huge concern in science, responsible for spend a lot of financial sources and scientific efforts worldwide. Commonly, it provides inaccurate results and leads the scientists to errors. IA is characterized by a false result due to the chemical structure of compounds and/or procedures used in the assays [1].

The most common features related to compounds that interfere in the assays are: Presence of reactive chemical groups (as for example, aldehydes), compounds that alter antibody binding or those which modify the concentration of the analytes, lack of solubility and precipitation of substances in the medium used in the assay, emission of fluorescence by compounds, promiscuous effects by activation of several targets simultaneously, among others [2-4].

Natural products have a privileged status in drug discovery exhibiting a wide range of biological activities. IA due to natural products is a reality in different laboratories worldwide; therefore, researchers to avoid false results must adopt strategies. The presence of chemical reactive groups that interfere in biological assays is frequent for natural products. Recently, Bell describes some chemical structural features associated with interference in assays including: catechols, quinones, phenolic Mannich bases, hydroxyphenylhydrazones, β-lactam, peroxide, epoxide and disulfide, or enone [5].

The interference of catechol groups in the bioassays occurs through different mechanisms, including chelation of metals and redox activation [5]. Not only catechols, but also phenolic groups present in natural products can contribute to false results in the assays. Examples of phenolic compounds with promiscuous activity include resveratrol, capsaicin, epigallocatechin-3-gallate (EGCG), curcumin and genistein. For these molecules, it was found their ability to cause membrane perturbation [6]. This deleterious effect can activate different pathways in the cells justifying the wide range of biological activities. Therefore, in order to minimize promiscuous effect due to membrane perturbation some assays are recommended, such as: gA single-channel electrophysiology, gA based fluorescence assay, MscL fluorescence assay, KV2.1 and $Na_V$ electrophysiology and molecular dynamic simulation [6]. EGCG is another example of natural product, which interfere in assays due to at least two effects: a) Oxidation to orthoquinones that binds covalently to proteins; and b) Ability to perturb membranes. Despite of these well-known effects, it is extensively reported in the literature as a 'promising' compound in unrelated cell-based assays and target-based assays.

Quinones are present in several natural products including menaquinone, menadione, mitomycin C, daunorubicin, mitoxantrone, thymoquinone among others. For these quinones, the deleterious effect mechanisms are due to its redox-activation and the presence of Michael acceptor (α,β-unsaturated carbonyl)-that reacts and binds covalently with cellular structures. Even for those compounds, which quinone function is included in cycles, is possible to observe these effects. In addition, quinone derivatives use to be fluorescents interfering directly in some bioassays. Thymoquinone, for example, was initially developed as anticancer compound. This natural product has exhibited high redox activity and ability to act as Michael acceptor. During clinical trials thymoquinone failed to show the desired pharmacological effect [7]. Despite of daunorubicin and mitomycin C are approved drugs, no confusion should be done between hits and drugs containing a quinone scaffold, because for hits in general the potency is observed at micromolar level.

Another example of natural product able to interfere in assays widely reported in the literature is curcumin. Even after primary studies suggesting its use for several diseases, Food and Drug Administration (FDA) do still not approve this compound as drug for human use. It has been reported that curcumin is an unstable molecule that chelates metals, labels proteins, exhibit redox-activity and perturb membranes [6,8].

Natural products, as reactive compounds, typically modify protein residues or interact with reagents in the assay through oxidation of sulfur in cysteine residues, act as Michael acceptor through nucleophilic substitution, form disulfide bonds by reaction of two thiol-containing molecules, among others. The presences of certain structure/chemical features in natural products must be comprehend as a 'red flag', which demand additional studies before being discarded as a no-promising compound.

Recently, we have discussed about the importance to identify Pan-Assay Interference Compounds (PAINS) in bioassays [9]. The use of filters such as REOS (Rapid Elimination of Swill) through computational chemistry allows the identification of 'red flag' scaffolds and minimizes the number of false-positive results using natural products in bioassays [10,11].

In summary, natural products interfering in bioassays is a reality in laboratories worldwide and the recognition of structural/chemical features is essential for biochemists and pharmacologists in order to minimize the publication of false-positive molecules. Currently, the use of orthogonal assays and PAINS filters are recommended to avoid the identification of a wrong prototype [12].

## Acknowledgments

Authors are thankful to Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP) (Process: 15/19531-1,14/06755-6,14/03945-9 and 13/23461-3).

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***Corresponding author:** Jean Leandro dos Santos, Faculty of Pharmaceutical Sciences, UNESP-The Universidade Estadual Paulista, Highway Araraquara Jau Km 01, 14801-902, Araraquara, SP, Brasil, Tel: +55-16-33016972; Fax: +55-16-33016960; E-mail: santosjl@fcfar.unesp.br

**Received** May 26, 2016; **Accepted** May 28, 2016; **Published** June 04, 2016

**Citation:** Dutra LA, Melo TRF, Chelucci RC, Chin CM, Santos JL (2016) The Paradigma of the Interference in Assays for Natural Products. Biochem Pharmacol (Los Angel) 5: e183. doi:10.4172/2167-0501.1000e183

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