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Pan-Assay Interference Compounds (PAINS): Warning Signs in Biochemical-Pharmacological Evaluations

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The identification of artifacts as being hit compounds by pharmaceutical companies and academy is not uncommon.Currently the term PAINS (pan-assay interference compounds) has emerged to described apparent bioactive molecules that are able to interfere in readouts through interaction with unrelated biological targets and/or testing methods [1]. Doubtless, the great inconvenience to develop 'false-hits' is the waste of timeand financial source spent with these compounds.

PAINS include around 400 structural classes and constitute 5-12% of all molecules in a typical academic screening library [1]. These compoundscould interfere in readouts by multiple ways including: nonselective reactivity with proteins [2] fluorescence [3], cysteine oxidation [4], aggregation [5], redox activity [6], membrane disruption [7] and chelation [8] among others.PAINS are commonly reported in the literature as promising hits against different proteins; however, an accurate look in chemical structure can help biochemists and pharmacologists to identify these structures before testing them.

The most frequently PAINS, reported in the literature, are: ene-rodhanine, phenol-sulphonamides, hydroxyphenyl hydrazones, isothiazolones, toxoflavin, curcumin, enones, quinones and cathecol [1]. Rodhadine, as for example, is extensively reported as promising bioactive compound; however, rodhanine and its derivatives could undergo light-induced reactions and modify covalently some proteins [9]. Phenol-sulphonamides derivatives are examples of unstable compounds which structure can cleave and interfere in the test. In addition, this scaffold could alterthe redox cycle and act as covalent modifier of proteins. Hydroxyphenyl hydrazones are known by its ability to sequester metal ionand inactive proteins. Isothiazolones can react with proteins covalently and modify its activity. Toxoflavin produces hydrogen peroxide and alter the function of some proteins. Curcumin is a covalent modifier compound that disrupts membranes. Enones, quinones and cathecol are covalent modifiers that alter redox cycle and promote ion metal sequester. Some phytochemicals such as resveratrol and genistein are examples of natural products that interfere in a series of biochemical assays [1].

In a recent paper, Dahlin and co-workers characterized several thiol-reactive chemotypes that interfere in assays and demonstrate promiscuous enzymatic inhibition [10]. The authors have confirmed by protein mass spectrometry and ALARM NMR that all these chemotypes react covalently with cysteine residues on multiple proteins. The 'bad' chemotypes/scaffold identified by authors include: benzothiadiazole/ benzofurazan; 1,2,4-thiadiazoles; succinimides, maleimides and *p*-hydroxyaryl sulfonamide. In addition, they proposedtwo main methods to identify nonselective cysteine reactivity: knowledge-based and experimental-based methods. Knowledge-based methods include substructure filters, computational calculations (i.e. frontier molecular orbital (FMO) calculations) and literature and database research (i.e. Pubchem). Experimental-based methods such as compound-target dilution, determination of activity time-dependence, redox-assays

(i.e. resazurin), direct detection (i.e. HPLC-MS) among others can complement the search for 'bad' molecules. If some PAINS is identified, it must be removed from investigation or be more rigorously evaluated using two or more experimental methods in order to avoid false results.

Researchers are encouraged to check database such as Sci Finder, Reaxys, BadApple or PubChemfor investigate if the chemical structures are notorious known for interfering in biological assays. Some functional groups that are considered warning sign and must be avoid in a chemical structure include alkyl and acyl halides, aziridines, anhydrides, alkyl sulfonates, isocyanates, peroxides, triflates, carbodiimide, disulfides, thiols, epoxides, aziridines, acyl and sulfonyl cyanides, aldehydes, reactive Michael acceptor, ketenes, carbamic and boronic acids, fluoro pyridines, nitro-aromatic and heteroaromatic, betalactones, betalactams, activated esthers and imines, hydrazines, cyclohexadiene and hydroxy arylaniline [11].

The use of filters to eliminate PAINS in biochemical and pharmacological assays are also recommended. Using computational chemistry (i.e. Tripos Software - Sybil 8.0), Baell and Holloway described new filters for removal PAINS from biological assays. These authors also include new motifs as PAINS such as phenolic Mannich bases, hydroxyphenyl hydrazones, alkylidene barbiturates, Alkylidene heterocycles, 1,2,3-aralkylpyrroles, activated benzofurazans and 2-amino-3-carbonylthiophenes [11]. Curpăn and co-workers also reported the description of filters for computational chemistry. The authors used density functional theory (DFT) for identification of PAINS [12].

In summary, this editorial demonstrates that PAINS must be recognized and avoided in biochemical and pharmacological assaysin order to avoid false results. The combination of knowledge-based methods (i.e. computational filters) and experimental-based methods can minimize this problem and increase the results accuracy.

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