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Identification by Inverse Virtual Screening of magnolol-based scaffold as new tankyrase-2 inhibitors

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Dedicated to the memory of our dear colleague and friend Carmela Spatafora.

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ABSTRACT

The natural product magnolol (**1**) and a selection of its bioinspired derivatives **2–5**, were investigated by Inverse Virtual Screening in order to identify putative biological targets from a panel of 308 proteins involved in cancer processes. By this *in silico* analysis we selected tankyrase-2 (TNKS2), casein kinase 2 (CK2) and bromodomain 9 (Brd9) as potential targets for experimental evaluations. The Surface Plasmon Resonance assay revealed that **3–5** present a good affinity for tankyrase-2, and, in particular, **3** showed an antiproliferative activity on A549 cells higher than the well-known tankyrase-2 inhibitor XAV939 used as reference compound.

1. Introduction

Inverse Virtual Screening (IVS) aims to identify putative macromolecular targets by computer-aided methodologies.[1,2] In this field, an *in silico* protocol has been developed by our research group,[3–5] consisting in a virtual screening of a single compound against a library of proteins by molecular docking and a normalization of the energy values as selection criteria for the macromolecule identification. It represents a rapid and cost efficient methodology for the identification of one or more potential biological targets for a small molecule. This approach, successfully applied in other cases reported in literature,[3,6–8] is particularly suitable for natural products, which are well-known to represent a precious source of new chemical skeletons endowed with a wide array of biological activities, whose biological targets are, most of the times, unknown. Recently, we have also demonstrated the applicability of our IVS protocol to the analysis of a synthetic compound library validating our *in silico* strategy for lead repurposing.[9] In this context, the biomimetic syntheses allow to broaden the chemical diversity of the natural scaffold, providing the possibility of increasing the bioactivities, as already reported in literature.[10–15]

The dimeric neolignan magnolol (**1**, Fig. 1) has been recently the focus of bioinspired investigations by some of us in order to develop a collection of structurally related derivatives, including compounds **2–5**; these were obtained employing both enzymatic dimerization in the presence of horseradish peroxidase (HRP), and regioselective *ortho*-hydroxylation with the environmentally benign reagent IBX (2-iodoxybenzoic acid).[13] In the present contribution, we investigated by IVS **1** and its derivatives **2–5**, succeeding in identifying new inhibitors of tankyrase-2. This macromolecule, along with the isoform 1, is a member of the PARP (poly ADP-ribose polymerases) family of proteins, acting as mono- or poly-ADP-ribosyltransferase and is involved in different biological processes such as telomere elongation and Wnt signal transduction pathway.[16,17] Owing to its involvement in different key cellular pathways, tankyrase-2 plays an important role in cancer, resulting as an attractive target for tumor treatment.[18–21]

2. Results and discussion

2.1. Inverse Virtual Screening

Briefly, our IVS protocol consists in molecular docking calculations of a small molecule against a panel of proteins, obtaining a predicted binding energy [V_0 (kcal/mol)] for each ligand-protein system. In parallel, we virtually test decoys, namely compounds showing molecular weights and H-bonds donors/acceptors similar to the investigated compound, against the protein panel. For each macromolecule, the

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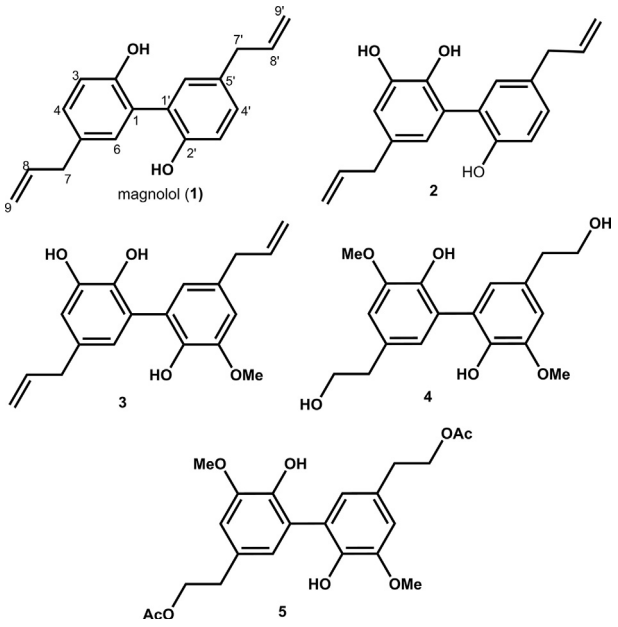

Fig. 1. Molecular structures of the natural product magnolol (**1**) and of its synthetic derivatives **2**–**5**.

average value V_R (kcal/mol) of binding energies of the decoys is calculated. The resulting ratio between V_0 and V_R is a dimensionless number V:

$$V = V_0/V_R \quad (1)$$

that is used to predict the most promising interacting targets for the case-study small molecule, ranking all the obtained V values from the highest to the lowest one. Such normalization is adopted to prevent the selection of false positives, as emerged by our previous studies.[4–9] In the present work, **1**–**5** were virtually screened on a panel of 308 models of proteins involved in tumor processes, and 162 decoys ("blanks" compounds) were used for the normalization of the docking results.[6] In details, for each small molecule (**1**–**5**), we obtained the predicted binding energies (see experimental sections) against the 308 protein models from molecular docking calculations. Each predicted value for a specific target was normalized with the corresponding averaged binding energy of the decoys. The normalized values of **1**–**5** were ranked from the highest (first position in the ranking) to the lowest one (last position in the ranking) obtaining an affinity profile on the whole protein panel (Tables S1–S5) for the small molecules. The next step was the analysis of docked conformations by visual inspection. In particular, we arbitrarily limited the analysis of docked outcomes for the targets ranked in the first 20 positions for each compound (**1**–**5**) classification in order to identify macromolecular candidates for the experimental assays.[9] From this analysis, putative macromolecular targets were identified for each investigated compound. By comparing the obtained results for **1**–**5**, we observed that each of the small molecules (**1**–**5**) targeted the proteins tankyrase-2 (TNKS2), casein kinase 2 (CK2) and bromodomain 9 (Brd9) at the same time. In particular, tankyrase-2 is ranked in the first ten best targets for **1**, **2**, **4** and **5** (Tables S1, S2, S4, S5). For the compound **3**, we found the tankyrase-2 at position twelve of the normalized values (Table S3). Similar considerations were made for casein kinase 2. Indeed, for **1**–**4** this protein is in the first ten positions (Tables S1–S4), whereas, for **5** it is classified at position fifteen (Table S5). The bromodomain 9 was found at position four and twelve

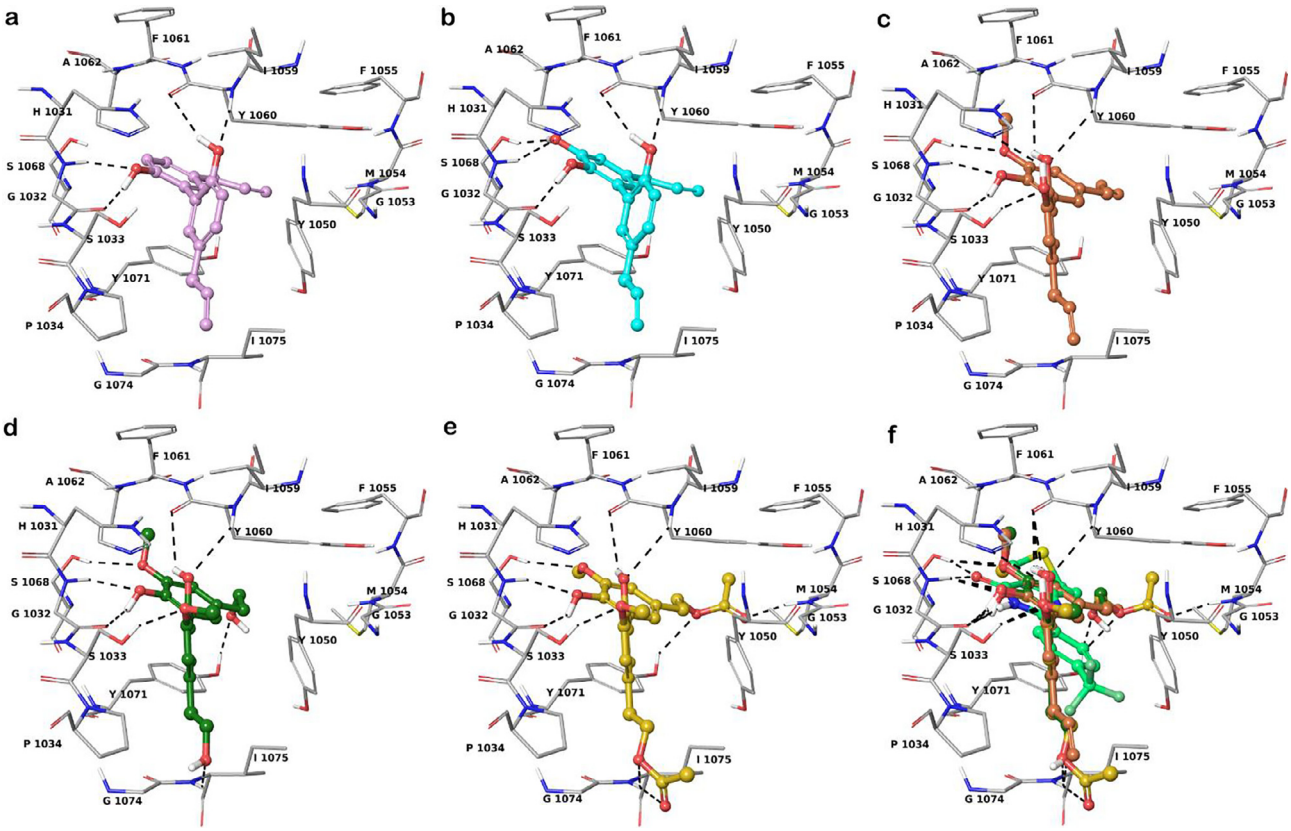

Fig. 2. Three-dimensional model of the interactions given by **1** (a), **2** (b), **3** (c), **4** (d) and **5** (e) with tankyrase-2. Superimposition of **3**–**5** with co-crystallized XAV939 (f, PDB ID 3KR8). The protein is depicted by tube (colored: C, grey; polar H, white; N, dark-blue; O, red; S, yellow). The small molecules are represented by sticks (pink for **1**, cyan for **2**, brown for **3**, dark green for **4**, kaki for **5**, aquamarine for XAV939) and balls (colored: C, as for the sticks; polar H, white; N, dark-blue; O, red; F, green; S, yellow). The dashed black lines indicate the hydrogen bonds between ligand and protein.