Bioorganic & Medicinal Chemistry 26 (2018) 2651–2673

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Design, *in silico* prioritization and biological profiling of apoptosis-inducing lactams amenable by the Castagnoli-Cushman reaction

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ARTICLE INFO

Article history:
Received 31 January 2018
Revised 16 March 2018
Accepted 16 April 2018
Available online 18 April 2018

Keywords:
Tumor suppressor
Transcription factor
Apoptosis induction
Protein-protein interactions
Virtual library
Antiproliferative activity

ABSTRACT

Five lactam chemotypes amenable by the Castagnoli-Cushman reaction of imines and cyclic anhydrides have been investigated for their ability to activate p53 tumor suppressing transcription factor thus induce apoptosis in $p53^{+}$ cancer cells. A virtual library of 1.07 million chemically diverse compounds based on these scaffolds was subjected to *in silico* screening first. The compounds displaying high docking score were visually prioritized to identify the best-fitting compounds, i. e. the ones which adequately mimic the interactions of clinical candidate inhibitor Nutlin-3a. These 38 compounds were synthesized and tested for apoptosis induction in $p53^{+}$ H116 cancer cells to identify 9 potent apoptosis-inducers (two of them exceeding the activity of Nutlin-3a) which belonged to four different chemotypes. The activation of p53 involved in the proapoptotic activity observed was supported by effective induction of EGFP expression in human osteocarcinoma U2OS-pLV reporter cell line. Moreover, the two most potent apoptosis inducers displayed antiproliferative profile identical to several known advanced p53 activators: they inhibited the growth of $p53^{+/+}$ HCT116 cells in much lower concentration range compared to $p53^{-/-}$ HCT116 cells.

1. Introduction

p53 protein is an important transcription factor whose activity (regulated by post-translational modifications such as lysine methylation and acetylation[1]) is central to maintaining genomic integrity and to tumor suppression.[2] Under normal circumstances (i.e. in the absence of cellular stress), p53 levels in the cell are kept relatively low by its proteasome degradation.[3] In order to become a client for the proteasome, p53 needs to be 'tagged' via ubiquitination. This process is promoted by MDM2 (murine double minute 2) regulatory protein which is an E3 ubiquitin ligase.[4] Thus, the current levels and the activity of p53 are to a large degree dependent on its interaction with MDM2 making it one of the most important protein-protein interactions in regulating p53 homeostasis.[5] At the same time, p53 is the most frequently mutated gene in human cancers.[6] Therefore, restoring the activity of p53 (dubbed the 'guardian of the genome'[7]) becomes a viable therapeutic approach to counterweighting the oncogenic mutation.[8] An obvious route to such an intervention would be to prevent the interaction of p53 with MDM2, which would suppress p53 ubiquitination and, as a result, its proteasome degradation.[9] This, in turn, would trigger apoptosis through the buildup of non-ubiquitinated p53 as well as other mechanisms and consequent elimination of the cancerous cell population.[10] Such a therapeutic rationale motivated numerous drug development programs which resulted in a number of investigational compounds[11] as well as candidates advanced as far as clinical trials.[12] Examples of such advanced compounds include: Roche's Nutlin-3a as well as RG7112, Sanofi's MI-77301 (initially discovered at the University of Michigan), and Amgen's AMG 232 (Fig. 1).[13]

In the discovery of new MDM2 inhibitors, the principal challenge is the poor druggability of protein-protein interactions (PPI) as a target for small molecule intervention in general.[14] Fortunately for those engaged in the discovery of new MDM2 inhibitors, the MDM2-p53 interface is very well characterized by crystallography.[15] Moreover, it is well established that three

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https://doi.org/10.1016/j.bmc.2018.04.036
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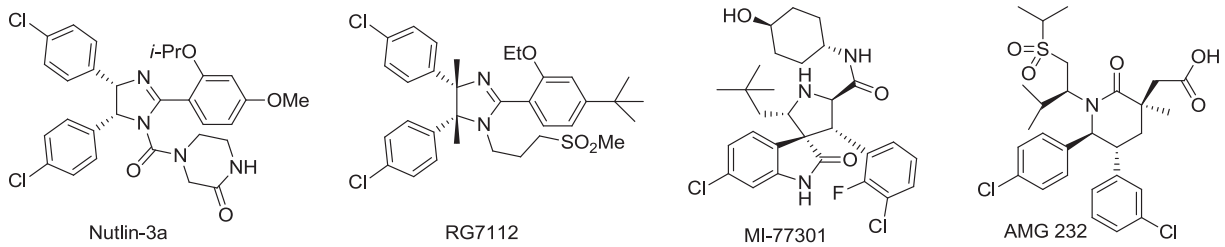

Figure 1. Advanced small-molecule inhibitors of the MDM2-p53 protein-protein interaction.

hydrophobic pockets on MDM2 (those accommodating L26, W23 and F19 of p53) are the principal 'hot spots'[16] for the inhibitor design. In fact, the crystal structure of Nutlin-3a with MDM2[17] shows that the molecular periphery of the former effectively addresses all the three hydrophobic pockets on the latter, thereby mimicking the three-prong interaction of MDM2 with p53 (Fig. 2).

In this work, we attempted to expose the groups that can fill the three hydrophobic pockets (HP1-3) off closely related lactam scaffolds **1**–**5** all of which are amenable by the Castagnoli-Cushman reaction (CCR)[18] followed by the amidation of the free carboxylic acid group in the initial CCR adducts (Fig. 3). The common chemistry underpinning scaffolds **1**–**5** was expected to eventually facilitate finding the optimal projection of the periphery elements. Moreover, the recent expansion of the CCR scope to include heteroatom-containing products (**3**–**5**)[19] would enable investigation of two alternative projection modes for the three-prong molecular periphery for the piperazin-2-ones **5** (as shown in Fig. 3). Additional consideration which prompted us to investigate scaffolds **1**–**5** in the context of disrupting MDM2-p53 PPI was the recently reported successful employment of related piperidin-2-one[20,21] and morpholin-3-one[22,23] scaffolds in Amgen's program aimed at the design of MDM2 inhibitors. Moreover, a recently published report on CCR-derived tetrahydroisoquinolonic acids acting as a 'Three-Finger Pharmacophore' and inhibiting the PPI in question[24] further supported the idea of investigating scaffolds **1**–**5** in the same context.

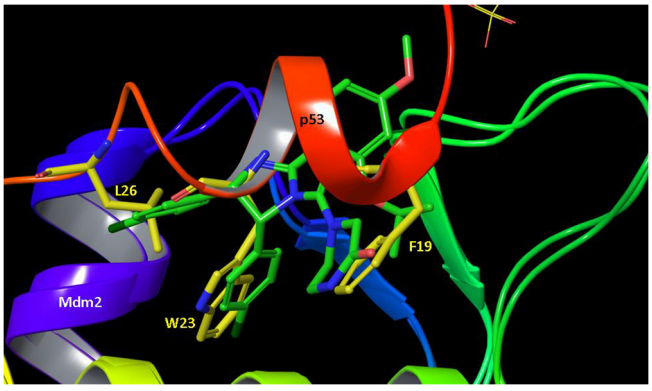

Figure 2. Interface of MDM2 (blue ribbon) and transactivation domain of p53 (red ribbon) (PDB 1YCR)15 and its superposition with Nutlin-3a (green) co-crystallized with MDM2 (PDB 4HG7).17.

2. Results and discussion

2.1. Virtual library design and docking prioritization

In order to establish the viability of the idea to use scaffolds **1**–**5** as the basis for proapoptotic p53 inducer design, we sought to generate a virtual library of compounds based on these scaffolds (as well as periphery reagents available in our stock for drug discovery) and perform prioritization of the potential inhibitors by docking them into the p53-binding region of MDM2. Since the CCR adducts can be obtained as either diastereomer,[24] it was decided to include both the *cis*- and *trans*-configured racemic compounds in the virtual library. Also, in order to explore the possibility

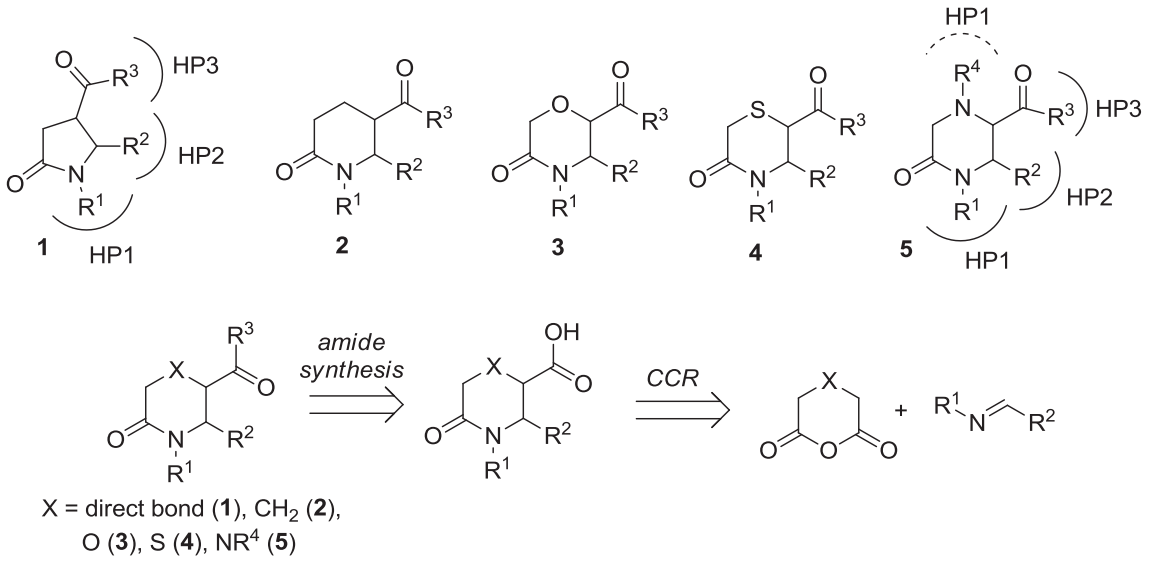

Figure 3. Lactam scaffolds **1**–**5** explored in this work (potential hydrophobic pocket filling envisioned for the compound's periphery shown) and their common retrosynthetic analysis.