Bioorganic & Medicinal Chemistry 24 (2016) 3174–3183

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

A combined ligand- and structure-based approach for the identification of rilmenidine-derived compounds which synergize the antitumor effects of doxorubicin

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ARTICLE INFO

Article history:
Received 20 March 2016
Revised 17 May 2016
Accepted 20 May 2016
Available online 24 May 2016

Keywords:
Drug design
Synthesis
Rilmenidine
Doxorubicin synergism
α_2-Adrenoceptors
Cytotoxic activity
Apoptosis

ABSTRACT

The clonidine-like central antihypertensive agent rilmenidine, which has high affinity for I_1-type imidazoline receptors (I_1-IR) was recently found to have cytotoxic effects on cultured cancer cell lines. However, due to its pharmacological effects resulting also from α_2-adrenoceptor activation, rilmenidine cannot be considered a suitable anticancer drug candidate. Here, we report the identification of novel rilmenidine-derived compounds with anticancer potential and devoid of α_2-adrenoceptor effects by means of ligand- and structure-based drug design approaches. Starting from a large virtual library, eleven compounds were selected, synthesized and submitted to biological evaluation. The most active compound **5** exhibited a cytotoxic profile similar to that of rilmenidine, but without appreciable affinity to α_2-adrenoceptors. In addition, compound **5** significantly enhanced the apoptotic response to doxorubicin, and may thus represent an important tool for the development of better adjuvant chemotherapeutic strategies for doxorubicin-insensitive cancers.

1. Introduction

Centrally acting hypotensive imidazoline derivatives, such as clonidine, rilmenidine and moxonidine, inhibit the activity of the sympathetic nervous system by activating α_2-adrenoceptors in the brain and possibly also via actions mediated by non-adrenergic I_1-imidazoline receptors (I_1-IR).[1–4] The prototypical agent, clonidine, has almost similar affinity for both types of receptors, whereas the newer antihypertensive agents moxonidine and rilmenidine bind more avidly I_1-IR than α_2-adrenoceptors. Possibly for this reason, they are less prone to elicit the typical side effects of clonidine, i.e., sedation, dry mouth and bradycardia. Recently, imidazoline derivatives have been also found to have other important biological effects not related to cardiovascular regulation, such as control of apoptosis and cell proliferation, both observed in vitro at micro- to millimolar concentrations (Fig. 1). Aceros et al.[5] reported that moxonidine, a moderately efficacious I_1-IR agonist, exerts proapoptotic effects in fibroblasts and antiapoptotic effects in cardiomyocytes. The imidazoline compound RX871024 induces cell death in insulin-secreting MIN6 cells.[6] S43126, an I_1-IR selective inhibitor of PC12 cell growth, caused considerable dose-dependent cell death, and apoptotic body formation after 72 h of treatment.[7] Our previous study demonstrated that rilmenidine induces significant membrane dissipation and deactivation of the Ras/MAP kinases ERK, p38 and JNK in cultured human leukemic K562 cells, thus exhibiting proapoptotic and antiproliferative effects.[8] However, our incomplete knowledge of I_1-IR signaling pathways and the lack of an I_1-IR crystallographic structure did not allow us to claim unequivocally which is the actual target through which rilmenidine exerts these effects. We have assumed that the action of rilmenidine is connected with its binding to the I_1-IR candidate nischarin and its interaction partner RAC1, a

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http://dx.doi.org/10.1016/j.bmc.2016.05.043
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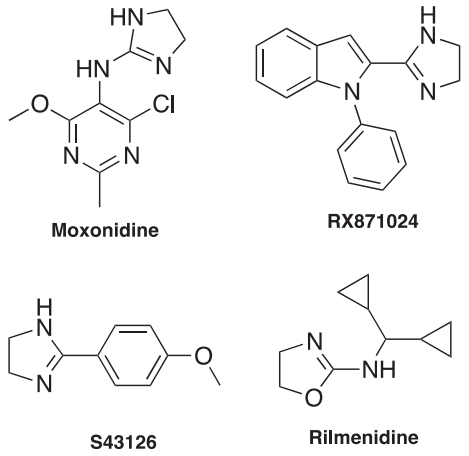

Figure 1. Structures of imidazoline compounds that regulate cellular apoptosis and proliferation in vitro.

member of the Rac subfamily of Rho guanosine triphosphatases (GTPases). Nischarin is a cytoplasmic protein[9] which besides rilmenidine binds numerous other imidazoline ligands and affects cellular signaling cascades controlling cell survival, growth and migration.[10,11] Along with the proapoptotic and antiproliferative effects, we discovered that the I_1-IR agonist rilmenidine rendered leukemic K562 cells, which are particularly resistant to many DNA-damaging chemotherapeutic agents, susceptible to the effects of doxorubicin.[8]

Doxorubicin, an anthracycline drug, is one of the most effective anticancer drugs ever developed, and although in clinical use for more than 30 years, it still plays an important role in the treatment of many hematological cancers (leukemias and lymphomas) and several types of solid tumors (carcinomas and sarcomas).[12] The therapeutic potential of doxorubicin is significantly limited by the risk of cardiotoxicity, which is dependent on the cumulative dose/treatment schedule and unpredictably evolves towards congestive heart failure. To avoid this serious complication, the maximum recommended cumulative dosage of doxorubicin has been set at 500 mg/m^2.[13] Regardless, doxorubicin cardiotoxicity may occur at lower cumulative doses if co-administered with other antitumor agents (e.g., paclitaxel, trastuzumab). These combined treatment modalities clearly offer improved response rates, but important cardiotoxicity may surface with cumulative doses of doxorubicin as low as 360–400 mg/m^2.[14,15] In the current study, we aimed at extending our earlier investigation on the rilmenidine binding site and I_1-IR candidate nischarin.[16] We used a combined ligand- and structure-based virtual screening approach to identify ligands that, similarly to rilmenidine, could establish effective interactions with nischarin and affect cell viability. In addition, by using nischarin as a target for structure-based virtual screening, we aimed at diminishing ligand binding to α_2-adrenoceptors. Exploring a large in-house virtual library, eleven hit compounds emerged as best candidates. Biological studies confirmed that the most active compound **5** indeed induces apoptosis and sustains the proliferation of K562 cells in a similar fashion as rilmenidine but with limited effects on α_{2A}-adrenoceptors. More importantly, we demonstrated that co-treatment with compound **5** and doxorubicin promotes substantial enhancement of the apoptotic responses of K562 cells compared to the single agents. This integrated medicinal chemistry study provides biologists and pharmacologists with a tool that may represent a promising starting point for the exploration of the I_1-IR pathway and for the development of improved adjuvant chemotherapeutic strategies for cancers with limited susceptibility to doxorubicin.

2. Results and discussion

Here we report the development of a novel virtual screening protocol for the identification of structurally diverse I_1-IR agonists with proapoptotic and antiproliferative activity. To limit the number of hits to be tested, the method included the use of an average quasi-valence number (AQVN)-similarity search, and ligand- and structure-based virtual screening approaches. This combination of techniques allowed us to select eleven different compounds for biological evaluation.

2.1. Chemoinformatic screening

Strong correlations between biological activities of organic molecules and the values of AQVN and electron ion interaction potential (EIIP) chemical descriptors related to long-range interaction properties have been observed.[17] In line with this principle, we proposed a simple criterion to discriminate the biologically relevant chemical space based on AQVN and EIIP. Previously, we demonstrated that 92.5% of about 45 million compounds from the PubChem database are homogeneously distributed within the AQVN interval (2.4–3.3). Recently, we reported the selection of HIV and Ebola inhibitors by means of AQVN- and EIIP-similarity searches together with other complementary VS approaches.[18,19] Here, we define an AQVN-based filter for the rapid in silico prescreening of large chemical libraries in order to identify novel rilmenidine analogues. For this purpose, we established the filter by expanding the area centered at rilmenidine's AQVN value (2.4828) to capture 20% of the AQVN space occupied by the I_1-IR agonists reported in the literature (Supplementary material). In this way we defined the rilmenidine AQVN space between 2.4296 and 2.5025. Filtering of compound sets described in Section 4 led to the pre-selection of 3005 compounds that were further investigated in a ligand- and structure-based virtual screening (Fig. 2).

2.2. Ligand- and structure-based virtual screening

A number of successful applications of FLAP (fingerprint for ligands and proteins) for the identification of G-protein-coupled receptor ligands have been recently reported.[20–23] However, to our knowledge, this is the first work reporting the application of VS approaches in order to disclose selective I_1-IR ligands with potential proapoptotic and antiproliferative activity. In this work we applied combined ligand- and structure-based virtual screening in order to take into account the variety of available chemical and biological information.[24] After AQVN-based filtering of the

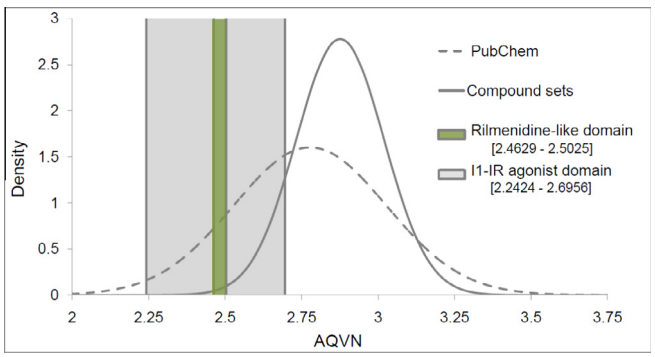

Figure 2. Compound distribution according to their average quasi-valence number (AQVN). 10.47% of the elements from the compound set described in Section 4 are within the I_1-IR agonist domain. The compound sets contain 3005 elements within the rilmenidine-like domain.