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Design, synthesis and biological profile of new inhibitors of multidrug resistance associated proteins carrying a polycyclic scaffold

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ABSTRACT

Following the identification of a novel polycyclic scaffold, leading to the previously reported potent P-gp modulator **1**, a small series of easily affordable derivatives bearing a properly selected nitrogen-containing but-2-ynyl side chain was now synthesized and tested to evaluate the MDR reverting activity on two different experimental models. All compounds proved not to be cytotoxic when tested alone and more potent chemosensitizers than the reference verapamil. Some of them showed remarkable effects in combination with doxorubicin, being able to induce apoptotic cell death due to their reverting activity. In particular, **2a** and **2c** could be regarded as non-toxic new potential chemosensitizers, being able to interfere with different ABC proteins. Moreover, the intrinsic cytotoxicity of compound **1** could broaden its employment as MDR modulator. These results also seem to confirm the polycyclic core of these compounds as a potential new pharmacophoric carrier in medicinal chemistry.

1. Introduction

Cancer is a hyperproliferative disease mainly characterized by transformation, dysregulation of apoptosis, angiogenesis, invasion and metastasis. Chemotherapy has been introduced into treatment strategies in the last few decades and it is well known that the effects of most classical antiproliferative agents could be related to an impairment in cell cycle progression [1]. The cell cycle is composed of a series of tightly integrated events that allow the cell to grow and proliferate. With the new progresses in understanding the basic molecular mechanisms underlying cell cycle regulation and apoptosis and how these processes are impaired in tumour cells, recent research has been addressed to identify molecules capable of directly interfering with the specific intracellular targets involved, such as cyclines, cycline-dependent kinases (CDKs), B-cell lymphoma-2 (Bcl-2), inhibitors of apoptotic proteins (IAPs), etc. [2–4]. Although the improvement in cancer therapies resulted in the discovery of new drugs and new therapeutic strategies, the onset of chemoresistance severely limits their area of application. In the last decades many efforts have been made to identify new experimental strategies to overcome this problem, involving the development of novel reversal agents (chemosensitizers) or drugs capable of killing resistant cells.

Several different mechanisms are involved in the development of resistance to chemotherapy, such as decreased uptake of water-soluble drugs, increased DNA damage repair, altered drug metabolism, reduced apoptosis, and increased ATP-dependent efflux of hydrophobic anticancer agents that enter the cells by diffusion through the plasma membrane. This last phenomenon is known as multidrug resistance (MDR) and is the major and still unsolved clinical problem in the treatment of cancer [5]. MDR can arise *de novo* or after exposure of cancer cells even to a single drug and is

Abbreviations: ABC, ATP-binding cassette; ATP, adenosine-5′-triphosphate; Bcl-2, B-cell lymphoma-2; CDKs, cycline-dependent kinases; DBP, drug-binding pocket; DXR, doxorubicin; IAPs, inhibitors of apoptotic proteins; MDR, multidrug resistance; MRP1, multidrug resistance protein-1; NBD, nucleotide-binding domain; P-gp, P-glycoprotein; GS-X pump, glutathione S-conjugate export pump; TMD, transmembrane domains.

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characterized by cross resistance to a series of structurally unrelated compounds with different subcellular targets. Well characterized mechanism contributing to MDR is the overexpression of membrane efflux pumps, belonging to the evolutionarily conserved family of ATP binding cassette (ABC) proteins, which transport anticancer drugs out of the cells so that effective intracellular drug levels are no longer reached. In humans, more than 48 MDR genes were identified from the ABC transporter superfamily, among which the most extensively characterized MDR transporters include P-glycoprotein (P-gp/ABCB1) and multidrug resistance associated protein-1 (MRP1/ABCC1) [6,7]. The constitutive expression of these proteins occurs in hematopoietic cells of peripheral blood, lung, testicle, placenta, brain, kidneys, adrenal gland, duodenum, heart, colon, and skeletal muscle [8]. These transporters play an important physiological role in detoxifying cells from both metabolites produced by normal cellular processes and exogenous toxic agents, such as chemotherapy drugs, which favours the resistance mechanism [9,10].

P-gp is a 170 kDa protein, classified, from a structural viewpoint, as a pseudosymmetrical heterodimer, where each monomer consists of six transmembrane domains (TMD), which is responsible for the recognition and transport of substrates, and one nucleotide-binding domain (NBD) for ATP binding and hydrolysis [11]. MRP1 is a 190 kDa protein consisting of 17 TMD having P-gp like cores, mainly located in the plasma membrane. MRP1 acts as an ATP-dependent glutathione S-conjugate export pump (GS-X pump), thus its MDR mechanism is entirely different from that of P-gp mediated resistance. However, many of the anticancer drugs which are substrates for P-gp are also substrates for MRP1 [12].

Nowadays, the most accepted strategy to overcome ABC-cassette mediated drug resistance consists in the co-administration of the anti-cancer drug with a reversal agent, capable of inhibiting its efflux from the cell. However, this strategy shows some drawbacks, related to both the functional role of these transporters in healthy tissues and the possibility of unpredictable drug–drug pharmacokinetics interactions. The P-gp drug binding site is indeed similar to the binding site of CYP3A4, leading to a possible concurrent inhibition of this enzyme, thus interfering with the intestinal or liver metabolism of the anticancer drug [13] A wide variety of natural and synthetic compounds with the properties of inhibiting P-gp have been described to date, from the calcium antagonist verapamil (Fig. 1), one of the first generation reversal agents, to the more specific potent third generation MDR modulators. Among these, the most promising proved to be tariquidar (XR9576) [14] and zosuquidar (LY335979) [15] (Fig. 1), which showed minimal pharmacokinetic interactions. They have entered clinical trials but to date are not clinically available.

However, the limited common features for the interaction of ligands with this protein have been postulated: a protonable nitrogen, aromatic rings, high lipophilicity and the ability of

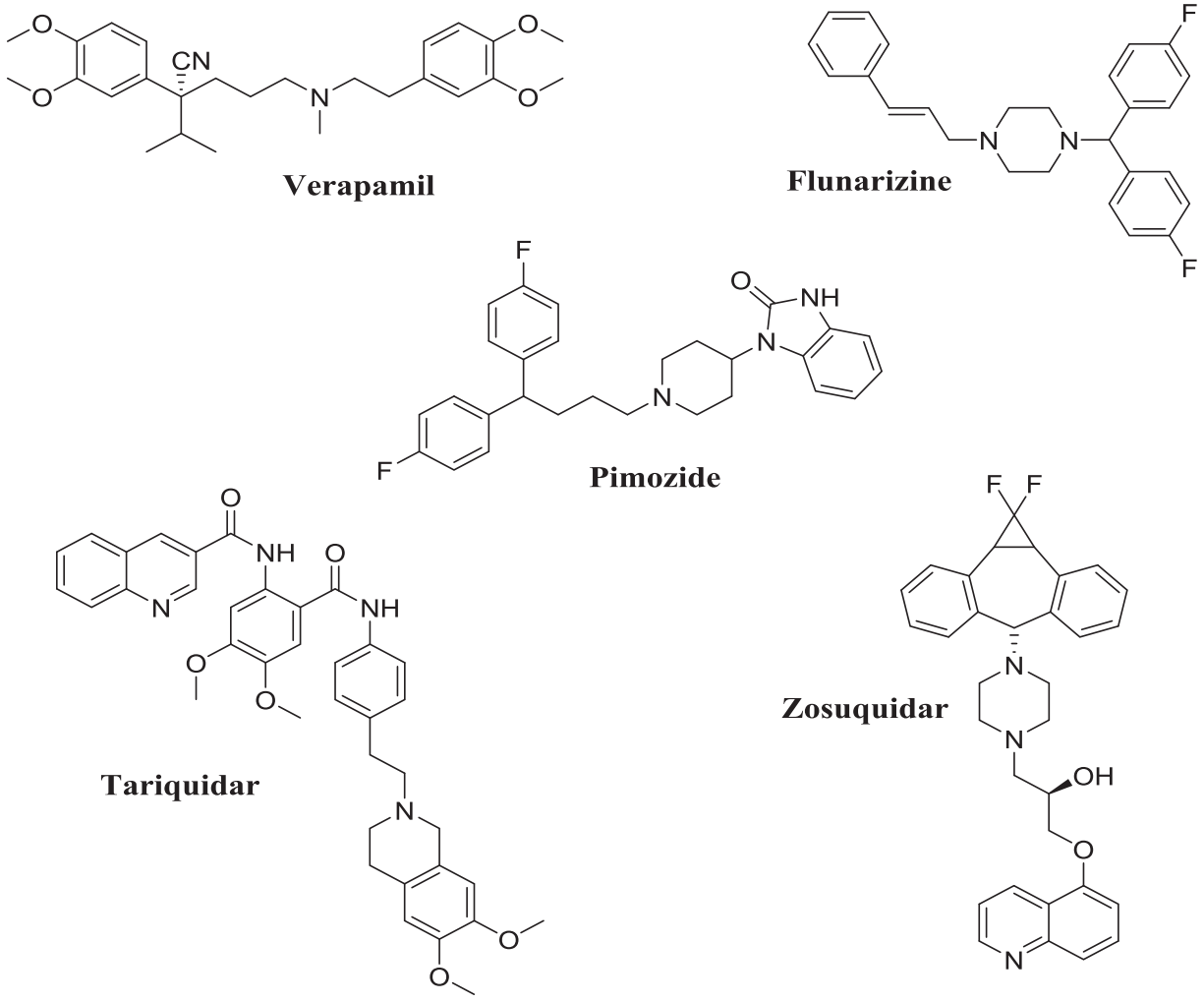

Fig. 1. Representative MDR reversal agents.