European Journal of Medicinal Chemistry 147 (2018) 7–20

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

Design and synthesis of new potent *N*,*N*-bis(arylalkyl)piperazine derivatives as multidrug resistance (MDR) reversing agents

Silvia Dei [a], Marcella Coronnello [b], Gianluca Bartolucci [a], Dina Manetti [a], Maria Novella Romanelli [a], Chatchanok Udomtanakunchai [c], Milena Salerno [d], Elisabetta Teodori [a, *]

[a] Department of Neuroscience, Psychology, Drug Research and Child's Health - Section of Pharmaceutical and Nutraceutical Sciences, University of Florence, via Ugo Schiff 6, 50019 Sesto Fiorentino (FI), Italy
[b] Department of Health Sciences - Section of Clinical Pharmacology and Oncology, University of Florence, Viale Pieraccini 6, 50139 Firenze, Italy
[c] Department of Radiologic Technology, Faculty of Associated Medical Sciences, Chiang Mai University, 50200, Thailand
[d] Université Paris 13, Sorbonne Paris Cité, Laboratoire CSPBAT, CNRS (UMR 7244), UFR-SMBH, 74 rue Marcel Cachin, 93017 Bobigny, France

ARTICLE INFO

Article history:
Received 31 October 2017
Received in revised form
18 January 2018
Accepted 28 January 2018

Keywords:
MDR reversers
P-gp modulators
Piperazine derivatives
Chemosensitizers
Doxorubicin-resistant human erythroleukemia K562 cells (K562/DOX)
Pirarubicin uptake

ABSTRACT

A series of 1,4-substituted arylalkyl piperazine derivatives were synthesized and studied with the aim to obtain potent P-gp-dependent multidrug-resistant (MDR) reversers. The new compounds were designed on the basis of the structures of our previous arylamine ester derivatives endowed with high P-gp-dependent multidrug resistance reversing activity. All new compounds were active in the pirarubicin uptake assay on the doxorubicin–resistant erythroleukemia K562 cells (K562/DOX). In particular, compounds bearing methoxy aromatic moieties showed fairly high reversal activities. The most potent compounds, **8**, **9**, **10** and **13**, were further studied by evaluating their doxorubicin cytotoxicity enhancement (reversal fold, RF) and the inhibition of P-gp-mediated rhodamine-123 (Rhd 123) efflux on the K562/DOX cell line. The results of all pharmacological assays indicated that the combination of a basic piperazine scaffold with arylalkyl residues allowed us to obtain very interesting P-gp modulating compounds. Two long-lasting P-gp pump modulators (**9** and **10**) were identified; they were able to inhibit remarkably the P-gp substrate rhodamine-123 efflux on the resistant K562/DOX cell line after 60 min. Overall compound **9** appeared the most promising compound being a potent and long-lasting P-gp–dependent MDR modulator.

1. Introduction

The success of the anticancer chemotherapy is frequently impaired by drug resistance that is the main defense mechanism that the tumor cells develop against chemotherapeutic drugs. Multidrug resistance (MDR) is a type of acquired drug resistance to multiple classes of structurally and mechanistically unrelated anticancer drugs [1]. The cells exhibiting MDR accumulate a lower intracellular concentration of drug as a result of an accelerated efflux of the antitumor agents mediated by an ATP-dependent process. The main mechanism responsible of this transport is the overexpression of ABC (ATP Binding Cassette) transmembrane proteins such as P-glycoprotein (P-gp, ABCB1), the multidrug-resistance-associated protein-1 (MRP1, ABCC1) and the breast cancer resistance protein (BCRP, ABCG2).

P-glycoprotein is the most studied of the ABC transporters. It is a membrane glycoprotein expressed in several important tissues and blood-tissue barriers where it plays important physiological roles as the regulation of the secretion of lipophilic molecules and the extrusion of exogenous toxic agents that enter the organism [2,3]. Nevertheless, P-gp is overexpressed in cancer cells as a result of an upregulation of the human gene expression MDR1 that causes an accelerated efflux of the chemotherapeutic drugs inducing the classical multidrug resistance (MDR) [4–6].

Circumvention of multidrug resistance through the inhibition of

Abbreviations: P-gp, P-glycoprotein; MRP1, Multidrug Resistance associated Protein 1; BCRP, Breast Cancer Resistance Protein; DOX, Doxorubicin; EDCl, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimmide hydrochloride; DMAP, 4-Dimethylaminopyridine; MTT, 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide; Rhd 123, rhodamine-123.

* Corresponding author.
E-mail address: elisabetta.teodori@unifi.it (E. Teodori).

https://doi.org/10.1016/j.ejmech.2018.01.092
0223-5234/

the functions of P-gp is considered an important field of investigation. MDR reversers (chemosensitizers) are P-gp modulators that administered in combination with cytotoxic agents, which are substrates of the efflux pump, could restore their efficacy in resistant cancer cells [7,8].

Drug possessing inhibitory properties have been and are actively being sought and many P-gp modulators, belonging to three generations of compounds have been identified [9–12]. A few of them have reached clinical trials [13,14], nevertheless, no drug has been approved for therapy. In fact, the development of safe MDR reversers is complicated by the P-gp physiological roles and the concurrent inhibitory action of these compounds on isoforms of cytochrome, like CyP3A4. Therefore, they could interfere with the pharmacokinetics of other substances, including the co-administered chemotherapeutic agents [15]. Thus, an ideal MDR reverser should be a potent and selective P-gp modulator without affecting the cytochrome activity.

The latest MDR reversing compounds, in addition to high potency and low toxicity, show a good specificity for P-gp displaying no pharmacokinetic interaction with chemotherapeutic drugs [16]. In particular, the third generation P-gp modulator Zosuquidar is a potent and selective P-gp inhibitor with almost no interaction with the multidrug-resistance-associated protein-1 (MRP1) or the breast cancer resistance protein (BCRP) and little effect on CyP3A4 [13].

Zosuquidar structure is characterized by the presence of a piperazine scaffold that is present also in other important MDR reversers as Dofequidar [17] (Chart 1). These compounds contain many of the features considered important for P-gp interaction, such as high lipophilicity, the presence of hydrogen bond acceptor groups, aromatic moieties and one or more protonable nitrogen atoms [18].

For many years our research group has been involved in the design and synthesis of several P-glycoprotein (P-gp) ligands with the aim to discover potent MDR modulators. Their structures are related to those of the tropane alkaloid pervilleine A, endowed with good MDR modulating activity and the first generation modulator verapamil [19] (Chart 2). Therefore these molecules are characterized by the presence of a basic nitrogen flanked at suitable distance by aromatic moieties The majority of these molecules are arylamine ester derivatives; likewise pervilleine A, they contain aromatic ester residues connected to a basic scaffold by linkers with different flexibility such as polymethylenic chains or cyclohexane rings (Chart 2, structure I). Most of these compounds have proven to be potent and efficacious P-gp-dependent multidrug resistance reversers [19–23]. The ester functions present in these molecules may be susceptible to enzymatic hydrolysis; therefore, to avoid any possible problem associated with the metabolic lability of this group, in the present study we reported the synthesis and preliminary pharmacological properties of a new series of derivatives carrying a piperazine nucleus as basic scaffold, and different arylalkyl groups on the two nitrogen atoms, avoiding the presence of ester functions in analogy to the structure of verapamil (compounds **1**–**13**, Chart 2).

These compounds were designed on the basis of the above mentioned structural requirements that are considered important for P-gp interaction (Chart 3). Therefore, aromatic substituents were chosen such as the cinnamyl (**a**), the 2-phenoxyethyl (**b**), the 2-isopropyl-2-phenylpentanenitrile (**c**) and the 4,4-diphenylbutyl (**d**) groups, that contain unsubstituted phenyl rings. The corresponding methoxylated moieties **g**, **h**, **i**, **l**, respectively, were inserted to evaluate the role of the hydrogen bond acceptor methoxy group, that is considered important for the MDR-reversing activity and is present in many well-known P-gp modulating compounds [9]. For the same reason the 3,4,5-trimethoxybenzyl group (**m**) was also inserted. The nucleus **e** was chosen to investigate the importance of the presence of fluorine, and the 9-methylanthracene nucleus **f** because it was present in compounds previously studied that proved to be very potent multidrug resistance reversers [19–21].

The reversal activity of the new compounds was evaluated in a preliminary screening by the pirarubicin uptake assay on doxorubicin-resistant erythroleukemia K562 cells. The pharmacological profile of the most potent compounds, **8**, **9**, **10** and **13**, was further studied by evaluating their doxorubicin cytotoxicity enhancement (reversal fold, RF) and the inhibition of P-gp-mediated rhodamine-123 (Rhd 123) efflux on the K562/DOX cell line.

In addition, an investigation about the stability of derivatives **8**, **9**, **10** and **13** was performed by evaluating the concentration of their solutions in phosphate buffer solution (PBS) at different incubation times. Moreover, the behaviour of these compounds in the presence of rat and human plasma was also studied to establish the actual concentration in biological conditions. In fact, their available concentration can be affected by media solubility or high affinity plasma protein binding.

2. Chemistry

The reaction pathways used to synthesized piperazine derivatives **1**–**13**, are described in Scheme 1. Reaction of *N*-*t*-BOC-piperazine [24] with the suitable arylalkyl halide in CH_3CN under basic conditions (K_2CO_3) gave compounds **15**–**19**. The arylalkyl halides are commercially available ((*E*)-(3-bromoprop-1-enyl)benzene) or were synthesized according to the literature (5-bromo-2-isopropyl-2-phenyl-pentanenitrile [25], 2-bromoethoxy benzene [25] and 9-(chloromethyl)anthracene [26]). The arylalkyl halide 5-(2-bromoethoxy)-1,2,3-trimethoxybenzene (**14**), used to synthesize compound **19**, was obtained by reaction of 3,4,5-trimethoxyphenol with 1,2-dibromoethane in anhydrous DMF, in a different way with respect to Drain [27] (Scheme 2). Finally, compound **20** was obtained by reaction of *N*-*t*-BOC-piperazine [24] with (*E*)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-ol [28] in the presence of CH_3SO_2Cl and anhydrous Et_3N, in anhydrous CH_2Cl_2. Cleavage of the protective group on compounds **15**–**20** was performed with CF_3COOH to obtain compounds **21**–**26**. These intermediates, together with 1-(3,4,5-trimethoxybenzyl)piperazine **27**, already described [29], were *N*-alkylated with the suitable arylalkyl halide and K_2CO_3 in CH_3CN to yield final compounds **1**–**13**. The arylalkyl halides are mentioned above or synthesized according to the literature (1-bromo-4,4-diphenylbutane [30], 1-bromo-4,4-bis(4-fluorophenyl)butane [31], 5-bromo-2-(3,4-dimethoxyphenyl)-2-isopropylpentanenitrile [32], 1-bromo-4,4-bis(4-methoxyphenyl)butane [33] and 5-(chloromethyl)-1,2,3-trimethoxybenzene [34].

3. Results and discussion

3.1. Modulation of pirarubicin uptake

The P-gp modulating ability of compounds **1**–**13** was evaluated on K562/DOX doxorubicin resistant cells that overexpress only the membrane glycoprotein P-gp [35–38]. K562 is a human leukemia cell line established from a patient with chronic myelogenous leukemia in blast transformation [39]. The uptake of THP-adriamycin (pirarubicin) was measured by a continuous spectrofluorometric signal of anthracycline at 590 nm ($\lambda_{ex} = 480$ nm) after cell incubation, following the protocols reported in previous papers [32,40]. The P-gp modulating activity of the studied compounds on the pirarubicin uptake test is expressed by: *i*) $[I]_{0.5}$, which measures the potency of the modulator and represents the concentration that causes a half-maximal increase ($\alpha = 0.5$) in the nuclear