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Original article

Multidrug resistance (MDR) reversers: High activity and efficacy in a series of asymmetrical *N*,*N*-bis(alkanol)amine aryl esters

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Dedicated to the memory of our colleague and friend Professor Serena Scapecchi prematurely deceased.

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ABSTRACT

As a continuation of our research on potent and efficacious P-gp-dependent multidrug resistance (MDR) reversers, several new *N*,*N*-bis(alkanol)amine aryl esters were designed and synthesized, varying the aromatic moieties or the length of the methylenic chain. The new compounds were tested on doxorubicin-resistant erythroleukemia K562 cells (K562/DOX) in the pirarubicin uptake assay, where most of the new compounds were shown to be active. In particular the asymmetrical compounds, characterized by two linkers of different length, generally showed fairly high activities as MDR reversers. Some selected compounds (isomers **15**–**17**) were further studied by evaluating their doxorubicin cytotoxicity enhancement (reversal fold, RF) on the K562/DOX cell line. The results of both pharmacological assays indicate that compounds **16** (GDE6) and **17** (GDE19) could be interesting leads for the development of new P-gp dependent MDR modulators.

1. Introduction

Multidrug resistance (MDR) occurs when cancer cells become resistant to chemotherapeutic drugs of different structure and mechanism of action, resulting in the failure of cancer treatment [1]. This kind of resistance is associated with the over-expression of a family of ATP-dependent transporter proteins that extrude the chemotherapeutic drug from the cells, lowering its concentration below that necessary for anticancer action. This hypothesis was first formulated 40 years ago [2] and confirmed when P-glycoprotein (P-gp) was identified and studied [3]. Further studies have shown that P-gp (ABCB1) is the most important member of a super family of ATP-dependent transporter proteins that includes BCRP (ABCG2) and MRP1 (ABCC1) as other representative players. P-gp, the best studied member of the family, is a highly permissive protein which transports a wide diversity of substrates. Intensive work on this family of transporters has shown that multidrug transporters are present in almost every cell where their physiological role seems to be that of controlling the traffic of lipophilic molecules and protecting the cell from toxic xenobiotics by extruding them from the cell [4,5]. ABC proteins and especially P-gp are expressed in many tissues, mostly in membrane barriers such as BBB (blood–brain barrier) [6]. Although a high resolution crystal structure of human P-gp is still lacking, fundamental information on its structure and mechanism of action has been collected from the resolution of the 3D-structures of the bacterial homolog Sav1866 [7] and murine P-gp (having 87% of homology with human

List of abbreviations: P-gp, P-glycoprotein; BCRP, breast cancer resistance protein; MRP1, multidrug resistance-associated protein 1; ABCB1, ATP binding cassette protein B1; ABCG2, ATP binding cassette protein G2; ABCC1, ATP binding cassette protein C1; DOX, doxorubicin; EDCI, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimmide hydrochloride; MTT, 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide.

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P-gp) [8,9] that has allowed the development of several useful homology models [[10] and therein cited literatures].

Since their discovery and the elucidation of the mechanism of action, P-gp and related proteins have been considered suitable targets for circumventing transporter-dependent MDR [11].

Verapamil and cyclosporin A were the early compounds showing P-gp modulating activity and, together with many other molecules, belong to the first generation of P-gp modulators. However, their toxicity prevented their clinical use and, at present, verapamil is only used as a standard in the search for new MDR reversing molecules.

A large number of compounds showing MDR modulating activity has been synthesized and studied since then (classified as second- or third-generation) [12–14] and several have reached clinical trials [15]. However, none has been approved for clinical use. Their low potency, unsatisfactory toxicity and adverse changes in the pharmacokinetic properties of the co-administered chemotherapeutic drug [16] have so far blocked their development and some experts have expressed skepticism about the future of MDR modulators [17,18]. Nevertheless, the search for new, safer, more potent and efficacious multidrug transporter modulators is still of interest, considering that such modulators seem to be useful for different purposes. In fact, P-gp has been recently considered an interesting target for CNS pathologies on the basis that it is an integral part of BBB [19,20]. It has been proposed that in Alzheimer's disease, the extrusion through the BBB membrane of toxic Aβ amyloid, often because of age-related reduction of P-gp, is impaired and that positive modulation of the transporter, for instance through P-gp enhancers, would be beneficial in preventing the disease [21,22]. However, the transport of Aβ amyloid by P-gp remains controversial [23,24]. On the contrary, the temporary negative modulation of the transporter could allow easier penetration of drugs into the brain to exert their action [25]. On the whole, there are several reasons for medicinal chemists to continue the search for molecules that could be used to modulate and study P-gp and related proteins.

In recent years we have studied several families of multidrug resistance modulators including *N*,*N*-bis(alkanol)amine aryl esters characterized by the presence of a basic nitrogen atom linked to two different aromatic ester portions by two identical polymethylenic chains of variable length as spacers. These compounds were designed based on the information that the presence of aromatic moieties and of one or more protonable nitrogen atoms is an important property for the P–gp interaction. Molecular flexibility was added as a further characteristic to allow the molecules to choose the most productive binding modes, within the large P-gp recognition site, which would result in a high affinity interaction. Actually, this approach provided good results since most of those compounds proved to be very potent MDR reversers [26,27].

Aiming to expand the structure–activity relationships and to optimize the potency and efficacy of compounds in this family, we have synthesized new derivatives (**1**–**11**, Chart 1) carrying other aromatic ester moieties. The spacers chosen, 3- or 5-methylenes long, were those showing the best results in previous studies [26,27].

The (*E*)-3-(3,4,5-trimethoxyphenyl)vinyl, 3,4,5-trimethoxy phenyl and anthracene moieties (**a**, **b** and **c**, Chart 1) that were present in the most potent of the previously synthesized compounds [26,27] were combined with new aryl moieties (**d**–**h**, Chart 1) chosen on the basis of the following rationale.

The lipophilic fluorenyl moiety (**d**) is present in potent compounds of the old sets studied [26,28]. The 3-(3,4,5-trimethoxyphenyl)ethinyl moiety (**e**) can be seen as analogous to the (*E*)-3-(3,4,5-trimethoxyphenyl)acrylic one (**a**) with altered

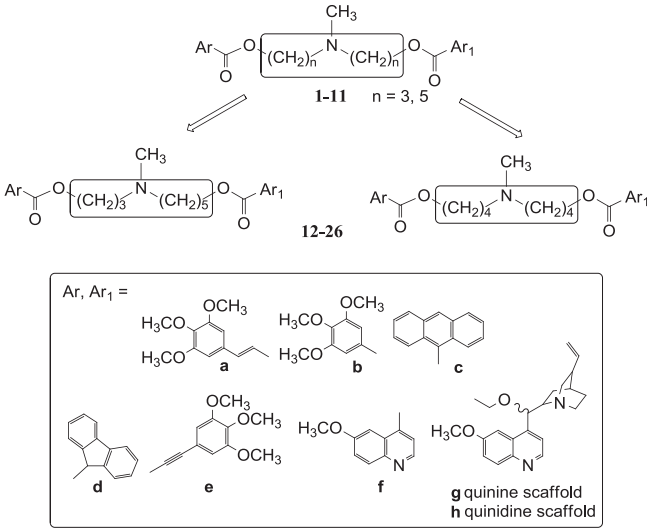

Chart 1. General structures of designed compounds.

geometry; this residue is also present in previously synthesized potent compounds [28,29].

Finally, the scaffold of quinine and quinidine (**g** and **h**, Chart 1) were chosen as being well known P-gp transport modulators [30,31]; since these residues were proved not to be productive, the 6-methoxyquinolinic moiety (**f**, Chart 1) was inserted to verify whether a simplified structure was better.

As a second approach we decided also to explore the consequences of varying the length of the spacers and of the relative position of the nitrogen in the chain, abandoning the symmetry of the linkers that characterized the previous series. To explore this point, we synthesized new derivatives of the *N*,*N*-bis(alkanol) amine series conserving the two aromatic ester portions (**a**–**e**, Chart 1), chosen by the same criteria as above, but connected by a chain of nine components, eight methylenes and one nitrogen atom, where the position of the nitrogen atom changes according to the length of the two spacers (3- and 5-methylenes long). The new compounds were compared to those having two spacers of identical length containing 4 methylenes that we synthesized in the present work for the first time (**12**–**26**, Chart 1).

The reversal activity of the new compounds was evaluated by the pirarubicin uptake assay in doxorubicin-resistant erythroleukemia K562 cells; this assay was used as preliminary screening. The compounds that showed the best activity in this test, the set of isomers **15**–**17**, were further studied by evaluating their doxorubicin cytotoxicity enhancement (reversal fold, RF) on the K562/DOX cell line.

2. Chemistry

The reaction pathways used to synthesize the designed derivatives (**1**–**26**) are reported in Scheme 1. The haloesters **27**–**35** were obtained by esterification of the suitable haloalkyl alcohol (3-bromopropan-1-ol, 4-bromobutan-1-ol or 5-chloropentan-1-ol) with the commercially available (*E*)-3-(3,4,5-trimethoxyphenyl) acrylic acid, 3,4,5-trimethoxybenzoic acid or anthracene-9-carboxylic acid. The desired esters were obtained by transformation of the carboxylic acid in the corresponding acyl chloride by reaction with $SOCl_2$ in ethanol-free $CHCl_3$, or by treatment of the mixture of haloalkyl alcohol and acid with the activating agent EDCI in the presence of DMAP in anhydrous CH_2Cl_2 (for details, see the Experimental section). Derivatives **27**–**29** and **33** had already been described by our group [26,27]; compound **32** had been obtained