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1-[4-(2-Aminoethoxy)phenylcarbonyl]-3,5-bis-(benzylidene)-4-oxopiperidines: A novel series of highly potent revertants of P-glycoprotein associated multidrug resistance

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Abstract

The 1-[4-(2-aminoethoxy)phenylcarbonyl]-3,5-bis-(benzylidene)-4-oxopiperidines **5**–**8** are a novel cluster of highly potent P-glycoprotein dependent multidrug resistance (MDR) revertants. Using a concentration of 4 μg/mL, these compounds possess 11–43 times the potency of verapamil in reversing MDR in murine L-5178 lymphoma cells transfected with the human MDR1 gene. Structure–activity relationships reveal that the attachment of the *N*-aroyl group to various 3,5-bis(benzylidene)-4-piperidones is essential for MDR reversal to occur. In terms of potencies, the 1-piperidinyl group is the preferred terminal amine while the 4-methyl and 4-chloro substituents are the optimal groups for placement in the benzylidene aryl rings.

Keywords

Multidrug resistance; Structure1–activity relationships; Physicochemical constants; *N*-Aroyl-3,5-bis(benzylidene)-4-piperidones

The principal objective of our laboratory is finding chemical approaches to counteract the ravages caused by cancer. A recent emphasis has been directed to finding compounds which reverse the vexatious problem of multidrug resistance (MDR).[1,2] The study described herein reveals the remarkable potencies of a novel series of P-glycoprotein (P-gp) associated MDR revertants namely the 1-[4-(2-aminoethoxy)phenylcarbonyl]-3,5-benzylidene-4-oxo-piperidines.

The problem of drug resistance occurs in many tumours and leads to an increased drug efflux from the neoplasms causing decreased intracellular drug concentrations thereby reducing the effectiveness of anticancer drugs. The mechanism of drug resistance is multifactorial but principally it is due to the overexpres-sion of P-gp, which is a member of

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the ABC (ATP-binding cassette) family of transporters.[3] P-gp is a 170 kDa membrane protein which behaves as a drug efflux pump with a very broad spec-ificity, and appears to act from the intracellular leaflet.[4] In humans, this protein is encoded by the *mdr1* and *mdr3* genes. While the MDR3 glycoprotein can bind to some of the substrates and inhibitors of MDR,[5] the low rate of this transport process usually makes it undetectable.

A number of studies revealed the increased potencies of anti-cancer drugs when administered with MDR revertants.[6,7] These chemosensitizers act in different ways, namely by binding to P-gp, inhibiting the efflux of the anticancer drugs from the tumours and reducing the binding of cytotoxins to P-gp.[8,9] In addition these compounds may reduce P-gp synthesis and/or inhibit MDR gene expression. A number of MDR modulators have pronounced bioac-tivities of their own such as verapamil and cyclosporine A,[10] which limit their clinical usefulness while most inhibitors of MDR are transporter substrates thus requiring high concentrations to overcome MDR.[11,12] To the best knowledge of the authors, these severe limitations have resulted in there being no clinically available MDR reversal agents to date and hence such medication is urgently required.

The design of a novel cluster of MDR revertants was made as follows. First, the common molecular features of a number of MDR modulators are their hydrophobicity, having two aryl rings and atoms capable of hydrogen bonding as well as bearing a positive charge at neutral pH.[13,14] Second, in terms of specific groups to be incorporated into the candidate MDR revertants, previous investigations from this laboratory revealed that various compounds which contain the 1,5-diaryl-3-oxo-1,4-pentadienyl moiety reverse MDR.[1,2] In addition, the 4-(2-aminoethoxy)phenyl substituent is present in the established MDR modulators amiodarone **1**[15] and toremifene **2**.[16,17] These general and specific molecular features were incorporated into the design of the candidate MDR revertants, whose general structure **3** is presented in Figure 1. The decision was made to evaluate the effect of placing different R groups in the arylidene aryl rings as well as varying the basic centre X. The compounds in series **5**–**9** were prepared by a previously reported methodology[18] and the synthetic chemical route is summarized in Figure 2. Since the 4-piperidones **4a**–**d** possess some of the molecular features found in series **5**–**9**, their assessment for MDR reversal properties was undertaken.

The compounds were assessed for MDR revertant properties using a literature method.[19] This assay employed murine L-5178 lymphoma cells transfected with the human MDR1 gene and in these cells the levels of P-gp are substantially higher than in the parental cells.[20] The concentrations of rhodamine 123 were measured in treated and untreated transfected and parental cells and the ratios of the fluorescence intensities are referred to as fluorescence activity ratio (FAR) values. Since MDR is due, *inter alia*, to an increase in the efflux of a compound from cells, a FAR value of greater than 1 indicates that reversal of MDR has taken place. All of the compounds were assessed using concentrations of 4 and 40 μg/mL and the data are presented in Table 1.

On a few occasions, the FAR values are lower at 40 μg/ml than when the lower concentration is used. This observation has been observed previously.[1] A possible explanation for this phenomenon is that when the concentration of a compound is elevated,

although binding to P-gp continues, other behavioural mechanisms are activated which expedite the extrusion of cellular contents. However the FAR values using 4 μg/mL will be considered since, with the exception of **8b** and **9b**, the higher concentration of compounds did not lead to substantial increases in the FAR data. The following observations were made from the MDR reversal experiments. First, MDR-reversal was displayed by all of the compounds in series **5**–**8** and the FAR values of these compounds ranged from 11 (**5a**) to 43 (**7b**) times that of the established MDR-reversing agent verapamil. These data clearly indicate that the incorporation of the 4-(2-aminoethoxy)phenyl carbonyl group into series **4** leading to series **5**–**8** generated a novel class of potent MDR-reversal agents. In general, the quaternary ammonium compounds **9** were inactive in this bioassay. Second, the optimal basic centre in series **5**–**8** was considered and the data presented in Table 1 reveals that the presence of a 1-piperidino group in series **7** is greatly preferred. Thus the average FAR values for the compounds in series **5**–**8** are 72.6, 87.8, 151 and 70.9, respectively. The pK_a values of dimethylamine, diethylamine, piperidine and morpholine are 10.73, 10.84, 11.12 and 8.50, respectively,[21] revealing that under the conditions of the bioassay, all of these compounds exist principally as ions.[22] The solvent-accessible surface area (SASA) figures for the protonated dimethylamino, diethylamino, piperidino and morpholino species are 183.8, 233.0, 234.6 and 224.1, respectively.[23] Thus apart from the smaller SASA figure for the dimethylamino group, neither the basicity nor the size of the amino group appears to govern the magnitude of the FAR values. However the biodata generated suggest that development of analogs of series **7** should take place in which the terminal basic group is a heterocycle containing one nitrogen atom such as hexamethyleneimine or heptamethyleneimine. Third, the substituents in the arylidene aryl rings were examined in terms of MDR-reversing potencies. In each of the series **5**–**8**, greater potencies were noted with the compounds bearing 4-methyl and 4-chloro substitutents. This observation may have been due to the π and molecular refractivity (MR) values of these groups. The π constants for hydrogen, methyl, chloro and nitro group are 0.00, 0.56, 0.71 and −0.28, respectively,[24] indicating the greater hydrophobicity of the methyl and chloro substituents. The MR figures of hydrogen, methyl, chloro and nitro groups are 1.03, 5.65, 6.03 and 7.36, respectively,[24] suggesting that an optimal MR value may have been reached while smaller and larger MR figures detract from MDR-reversing properties. Thus groups which are lipophilic and have MR values of approximately 5–6 should be placed in the arylidene aryl rings, for example, the trifluoro-methyl substituent has π and MR values of 0.88 and 5.02, respectively.[24]

A number of studies revealed the high lipophilicity of various MDR-reversal agents[25,26]; for example, the calculated log*P* values of a number of 1,4-dihydropyridines which reverse MDR were in the region of 5.1–7.5.[27] The calculated log*P* values of the compounds in series **4**–**9** and verapamil are presented in Table 1. The average log*P* figures for the compounds in series **4**–**9** are 3.90, 5.14, 5.90, 6.05, 4.99 and 1.92, respectively. Thus the compounds displaying significant capabilities in reversing MDR, namely series **5**–**8**, have log*P* values in the region of 5–6. On the other hand, the compounds in series **4** and **9**, which are virtually bereft of anti-MDR properties, have lower log*P* values. However, linear and logarithmic plots between the FAR values and the log*P* figures of the compounds in series **5**–**8** did not reveal any correlation ($p > 0.05$).

The potential of these compounds as MDR revertants will be enhanced if bioactivity is displayed at lower concentrations than 4 μg/ml and reversal of MDR occurs in a different species and another neoplastic disease. Consequently, the most potent compound identified in this study, namely **7b** along with its analog which is bereft of a *N*-acyl group viz. **4b** was evaluated further. These results are presented in Table 2. A concentration of 0.4 μg/mL of **7b** but not **4b** demonstrated MDR-revertant properties using L5178/MDR1 cells. In addition, **7b** reverses MDR in Colo 320/MDR1 cells while **4b** displays marginal potencies. These data confirm the relative potencies of **4b** and **7b** and in particular emphasize the potential of **7b** as a MDR revertant.

A further issue to be resolved is whether the *N*-acylpiperidones described in this report are cytocidal to neoplasms which are multidrug-resistant. Consequently, several representative compounds were assayed for cytocidal activity towards L5178Y/MDR1 cells.[28] The ID_{50} figures for **6a**–**c** are 3.27, 1.56 and 1.53 μg/mL, respectively, while for **7a**–**d**, the relevant values are 3.28, 2.83, 4.17 and 2.15 μg/mL, respectively. Verapamil has an ID_{50} figure of 42 μg/ mL. This observation emphasizes further the importance of developing these cytotoxins, which possess MDR-revertant properties.

The MDR modulators are believed to bind to the transmembrane domains of P-gp which leads to inhibition of ABC transporters due to the induced conformational changes.[25] The functionally active conformation of P-gp depends on the integrity of the membrane bilayer in which P-gp is embedded. Due to the existence of 12 transmembrane domains of P-gp, the co-crystallization of MDR inhibitors and P-gp is not possible thereby precluding direct evidence of ligand binding.

A final consideration which demonstrates the importance of this seminal work is the very recent disclosure of the excellent tolerability of the compounds in series **4**–**8**.[29] Thus doses of up to and including 300 mg/kg of each of these compounds did not induce lethalities in a short-term toxicity study in mice. A few compounds were examined at lower doses in rats which also did not induce mortalities. However, most of the compounds in series **9** causing deaths in some of the mice and bearing in mind the lack of MDR revertant properties in general, suggest that formation of further quaternary ammonium analogs of **9** is not pursued.

In conclusion, this study has identified a novel class of P-gp associated MDR-reversal agents **5**–**8**. These compounds demonstrate high potencies which far exceed that of a reference drug verapamil. Optimal structural fragments in terms of potencies are the 1-piperidinyl group as the terminal base and 4-methyl and 4-chloro substituents are present in the arylidene aryl rings. Series **4** has the general molecular features of various MDR revertants, as well as possessing the 1,5-diaryl-3-oxo-1,4-pentadienyl group. However, the absence of the 4-(2-aminoethoxy)- phenylcar-bonyl group attached to the piperidyl nitrogen atom resulted in compounds displaying a lack of MDR reversal. Development of one or more of these molecules may produce a single drug candidate to treat P-gp mediated MDR cancers and structural modifications can be undertaken in the future in order to increase potencies still further. In this regard, various guidelines for amplifying the project have been obtained based on the physicochemical properties of these molecules.

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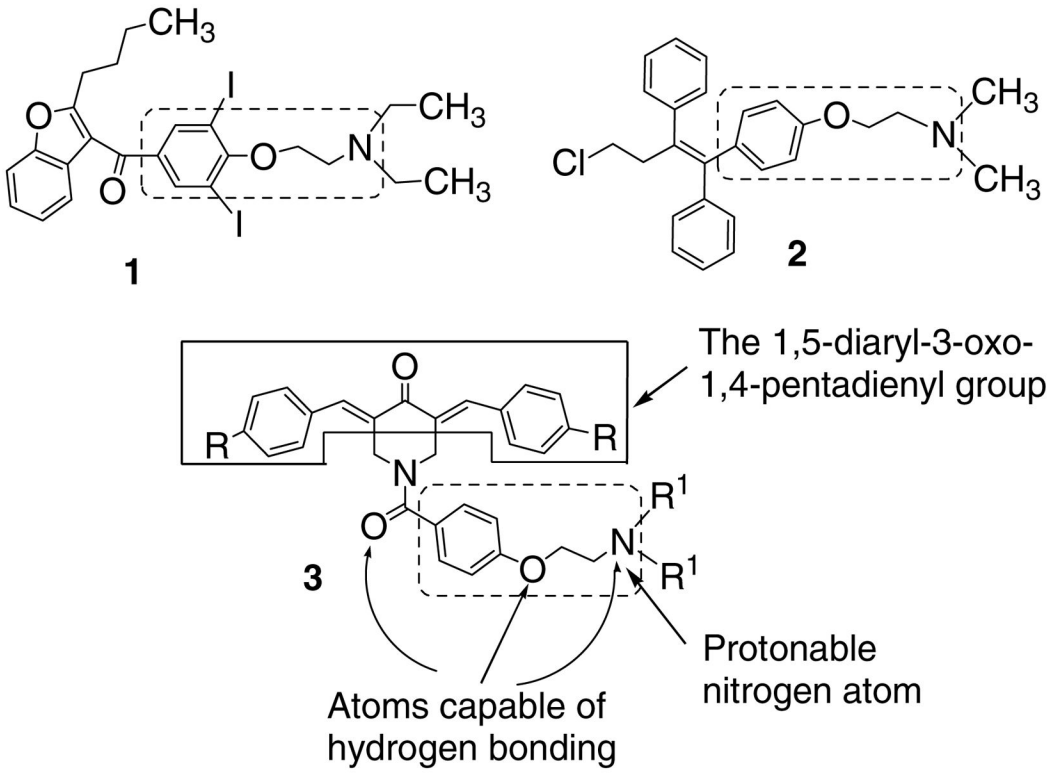

Figure 1.

The structures of amidarone **1**, toremiphene **2** and the general formula **3** of the candidate MDR revertants prepared in this study. The groups within the dotted lines in structures **1–3** are the common molecular features of some MDR revertants.

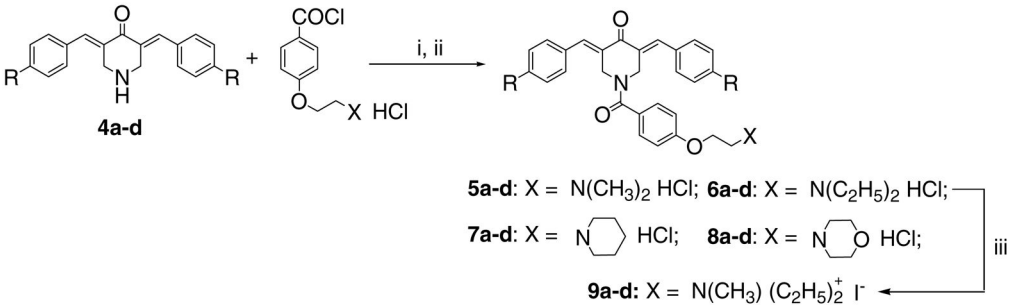

Figure 2.

Synthesis of the compounds **5–9**. The reaction conditions are as follows: i—CH_2Cl_2/ $N(C_2H_5)_3$, ii—HCl/ $(CH_3)_2CHOH$; iii—a, K_2CO_3; b, CH_3I/CH_3COCH_3. The nature of the R groups in series **4–9** are as follows, namely **a**: R = H; **b**: R = CH3; **c**: R = Cl; **d**: R = NO_2.

Table 1

MDR-reversing properties in murine L-5178Y/MDR1 lymphoma cells and log*P* values of the compounds in series **4**–**9** and verapamil

Compound	FAR value[a]		log*P*[b]
	4 μg/ml	40 μg/ml	
4a	1.58	2.00	3.38
4b	3.26	4.03	4.25
4c	1.25	0.91	4.71
4d	1.04	0.80	3.27
5a	45.5	51.6	4.60
5b	83.7	—[c]	5.50
5c	98.0	98.9	5.95
5d	63.0	3.23	4.52
6a	49.5	23.8	5.35
6b	106	66.6	6.25
6c	118	99.7	6.71
6d	77.5	14.1	5.27
7a	123	136	5.51
7b	179	146	6.40
7c	157	133	6.86
7d	145	51.5	5.42
8a	48.5	63.3	4.64
8b	95.8	124	5.34
8c	76.0	6.88	5.80
8d	63.3	10.9	4.36
9a	1.37	1.05	1.37
9b	1.93	114	2.27
9c	1.21	1.85	2.73
9d	1.03	1.22	1.30
Verapamil	4.2	—[d]	4.55

[a] The fluorescence activity ratio (FAR) values are the ratios of the fluorescent intensities of rhodamine 123 in treated and untreated murine L-5178Y cells transfected with human MDR1 gene.

[b] The log*P* values are of the unprotonated molecules and were determined using a commercial software package.[30]

[c] A concentration of 40 μg/ml of **5b** was toxic to the cells.

[d] Limitations of solubility precluded an assessment of verapamil at 40 μg/mL. The FAR value of this compound at 10 μg/mL is 5.61.

Table 2

Evaluation of **4b** and **7b** for MDR-revertant properties using murine L5178Y/MDR1 and human colo320/MDR1 cells

Compound	FAR value		
	L5178Y/MDR1 cells	colo320/MDR1 cells	
	0.4 μg/mL[a]	0.4 μg/mL[a]	4 μg/mL[b]
4b	1.00	1.24	2.42
7b	7.35	2.49	10.8

[a] Verapamil is inactive using this concentration.

[b] The FAR value of verapamil is 3.84 using a concentration of 10 μg/mL.