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Reversal of multidrug resistance by bis(phenylalkyl)amines and structurally related compounds

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Abstract. We have previously reported that multidrug (MDR)-reversal activity can be exerted by compounds in which two ring structures of certain types are connected by one alkyl bridge to a secondary or tertiary amine group. In the present investigation we studied the MDR-reversal activity of compounds in which the two ring structures were connected by separate alkyl bridges to the amine group. The structure-activity relationship of these compounds verified previous findings on the structural features that support MDR-reversal activity as well as the features that reduce such activity. In addition, the present study reveals additional chemical groups and ring structures that support MDR-reversal activity as well as those that reduce it.

Key words: Multidrug resistance – Bis(phenylalkyl)amines – Structure-activity relationship

Introduction

In recent years many compounds have been reported as potential reversing agents of multidrug resistance (MDR; for references, see [1, 7]). In most studies one or a few compounds were tested, and it is difficult to compare the relative efficacy of these compounds, especially as they were tested in different MDR cell lines. In a few studies attempts were made to elucidate the structure-activity relationships among such compounds (for references, see [2, 3]). These investigations, some computer-aided, revealed certain common structural features shared by many MDR-reversing compounds. However, in view of the structural diversity of the MDR-reversing agents, it is not certain that the analyses carried out with a relatively limited number of compounds have yet revealed all the structural features of the optimal MDR-reversing compound.

We have previously analyzed the relationship between the chemical structure of phenothiazines and related compounds and their ability to ameliorate MDR [3]. It was concluded that such activity was displayed by compounds containing two ring structures linked by a single alkyl bridge to a secondary or tertiary amine group. The MDR-reversal activity was enhanced by the presence of a carbonyl group. However, MDR-reversal activity has also been reported for many compounds that do not share these structural features. In fact, even in the verapamil molecule, the first and one of the most effective in vitro MDR-reversing agents, the two phenyl rings are connected to the amine by two separate alkyl bridges rather than by one bridge as found among the MDR-reversing agents of the phenothiazine type [8]. It was therefore expected that MDR-reversal activity might also be found among other compounds sharing a bis(phenylalkyl)amine structure. In the present paper the in vitro MDR-reversal activity of 311 such agents and related compounds is described and an effort is made to define further the structural features that are related to this activity.

Materials and methods

The 115 compounds presented in Table 1 were generously donated by the following suppliers (listed by compound number): 25 – Abbott Labs (Abbott Park, Ill.); 18, 51, 111, and 113 – Asta Pharma (Frankfurt, Germany); 110 – Labs A Bailly-SPEAB (Ivry/Seine, France); 98, 99, and 104 – Bayer AG (Wuppertal, Germany); 26 and 76 – Bristol-Myers Squibb (Princeton, N. J.); 73 and 75 – Duphar (Weesp, Holland); 102 – AB Hassle (Molndal, Sweden); 80 – Helopharm (Berlin, Germany); 19, 20, 23, and 27 – Hoechst AG (Frankfurt, Germany); 36–41, 47, 72, 114, and 115 – F. Hoffmann La Roche (Basle, Switzerland); 81 and 88 – ICI (Macclesfield, UK); 8, 10–13, 21, 96, 108, and 109 – Janssen Pharmaceutica (Beerse, Belgium); 43 – Jouveinal Labs (Fresnes, France); 22 – Kali-Chemie Pharma GmbH (Hannover, Germany); 29 and 54–71 – Knoll AG (Ludwigshafen/Rhein, Germany); 7 – Lipha (Lyon, France); 4 – McNeil Pharmaceutical (Raritan, N. J.); 6, 81–84, 92, and 112 – Marion Merrell Dow Inc. (Cincinnati, Ohio); 100 and 103 – Miles Inc. (West Haven, Conn.);

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93 – Norgine Ltd. (Oxford, UK); 24, 53, and 90 – Parke-Davis (Ann Arbor, Mich.); 101 – Pfizer Central Research (Groton, Conn.); 74 – Ravensberg GmbH (Konstanz, Germany); 2 and 5 – Rhone-Poulenc Ltd. (Dagenham/Essex, UK); 106 – Rhone-Pulenc Rorer (Collegeville, Pa.); 77 – Riom Labs-CERM (Riom, France); 30 and 105 – Sandoz Ltd. (Basle, Switzerland); 52 – Sanofi Pharma (Brussel, Belgium); 3 – Servier (Suresnes, France); 14–17, 50, and 78 – Sterling Research (Rensselaer, N. Y.); 97 – Synthelabo Recherche (Bagneux, France); 42 – Dr. K. Thomae GmbH (Biberach/Riss, Germany); 85, 86, 89, and 91 – Upjohn Co. (Kalamazoo, Mich.); and 32 and 107 – Wyeth-Ayerst Research (Princeton, N. J.). Compounds 1, 9, 28, 31, 33–35, 44–46, 48, 49, 79, 94, and 95 were purchased from Sigma-Aldrich Israel (Petach Tikva, Israel).

Our standard test system has been described elsewhere [6]. Briefly, P388 murine leukemia cells and a P-glycoprotein-containing MDR subline (P388/ADR) were maintained in RPMI 1640 medium supplemented with 10% fetal calf serum, 10 μ*M* 2-mercaptoethanol, penicillin base (50 IU/ml), and streptomycin (50 μg/ml). An inoculum of cells was transferred to fresh medium once every 4 days to maintain exponential growth. The sensitivity of both cell lines to a given drug was assessed as follows. Cells (1×10^6) were cultured in 10 ml medium in the presence of various drug concentrations (up to 100 μ*M*, or lower if limited by solubility). Once a day for 4 days the density of the cells was measured with a Coulter counter (Coulter, Harpenden, UK). The cell-growth rate was calculated from the slope of the log cell density versus time by linear regression analysis. The growth rate at each drug concentration was expressed as a percentage of the control growth rate (no drug). Dose-response curves were thus produced and used to determine the concentration of drug effective in inhibiting the growth rate by 50% (ED_{50}). In repeated experiments the standard deviation of this parameter was consistently $<10\%$ of the ED_{50} values obtained. The ability of a compound to ameliorate MDR was evaluated by comparing the ED_{50} obtained in P388/ADR cells incubated in the absence versus the presence of 0.2 μ*M* ADR. This ADR concentration was just below the concentration that produced a detectable growth-inhibitory effect on these cells (the ADR ED_{50} values in P388 and P388/ADR cells were 3.5×10^{-8} and 9×10^{-7} *M*, respectively).

We have previously shown that evaluation of the MDR-reversal activity of a compound using this experimental design is not inferior to that obtained using a design whereby the cells are incubated with increasing concentrations of ADR in the presence or absence of one subinhibitory concentration of the compound tested [4, 5]. Both experimental designs detected cytostatic synergism between the tested compounds and ADR with equal efficiency. The real advantage of the experimental design used in this study is the straightforward ability to compare MDR-reversal activity among the compounds tested. Moreover, this experimental design is substantially more economical.

Results and discussion

In the phenothiazines study we found that certain structural features interfered with MDR-reversal activity, including (a) the lack of a secondary or tertiary amino group, (b) the presence of a carboxyl group, (c) the presence of a single ring structure or the situation in which one or both rings were of the pyridine type [3]. In the present study, 93 compounds with such adverse features were tested and 83.4% of them had at best marginal MDR-reversal activity (ratio, <3) as defined in the legend to Table 1. Only 9 compounds (bucainide, pifarnine, TMB8, 3-methyl chloroquine, dibucaine, mefloquine, quinine, euprocin, and nicergoline) displayed good activity (ratio, ≥ 10 but <17). These data are not shown but are available on request.

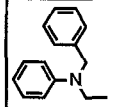
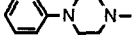
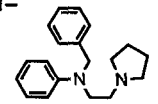
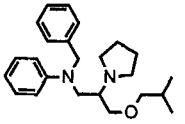
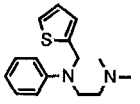
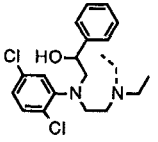
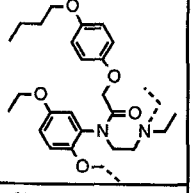
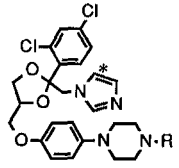
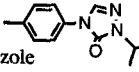
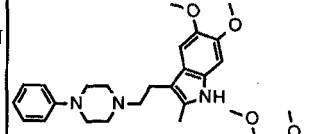
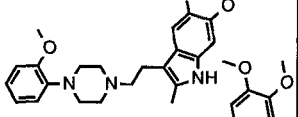
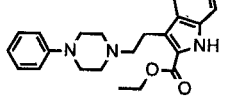
The structure-activity relationship (SAR) analysis of the other 218 compounds tested in the present study revealed additional structural features that reduced MDR-reversal activity and that were not noticed in the phenothiazines study. They include (a) the location of the amine group in an imidazole ring; (b) hydroxyl or primary amine function on the phenyl rings; (c) permanent charge; and (d) the situation in which one or both ring structures are a pyrrolidine, oxindole, isoindolinone, piperidine, isoquinoline, 1,2,3,4-tetrahydroisoquinoline, 2,3-benzofuran, 1-isobenzofuranone, 1,4-benzodioxan, benzimidazole, *N*-benzylbenzimidazole, pyrimidine, 4-phenyloxazole, thiazole, morpholine, 1,3,4-triazolo[1,5a]pyridine, 1,3,4-triazolo[1,5a]-5,6,7,8-tetrahydropyridine, 1,2,3-benztriazole, tetrazole, 2,3-dihydroindene, or naphthalene. In the present study, 103 compounds with these adverse features were tested and 66% of them produced an MDR-reversal ratio of <3. Only 5 compounds (isoconazole, R40500, tolnapersine, R6033, and nafiverine) yielded a ratio of ≥ 10 (but <14). These data are not shown but are available on request.

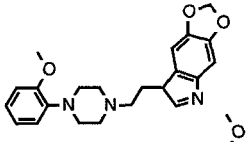
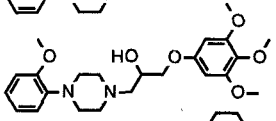
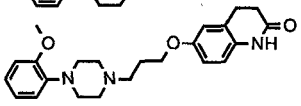
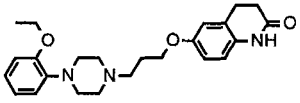
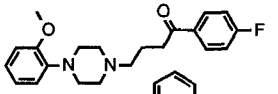
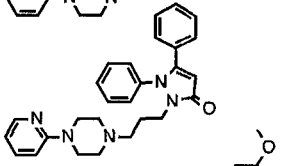
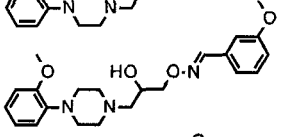
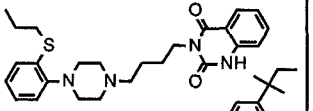
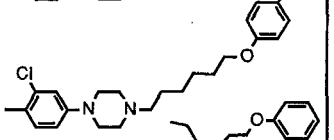
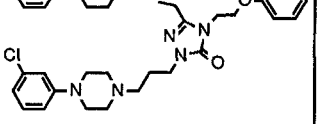
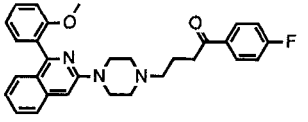
The results obtained for the remaining 115 compounds tested, which do not have the adverse structural features mentioned above, are shown in Table 1. The data in the table were entered according to similarities in molecular structure. For each compound tested, the ED_{50} value (expressed in micromolar concentration) obtained in P388 and P388/ADR cells are listed in columns A and B, respectively. For each compound, the ratio of the ED_{50} value obtained in P388/ADR cells in the absence of ADR to that measured in its presence (0.2 μ*M* ADR) is shown in column C. Therefore, the value in column C represents the ability of the compound to reverse MDR. The growth-inhibitory activity of almost every compound was also tested in drug-sensitive P388 cells in the presence of 0.01 μ*M* ADR; however, in no case was a >2-fold decrease in the ED_{50} value observed (data not shown). As shown in column C of Table 1, 20 compounds (17.4%) produced an MDR-reversal ratio of <3, 72 (62.6%) gave a ratio of ≥ 10, and 28 (24.3%) yielded a ratio of ≥ 30.

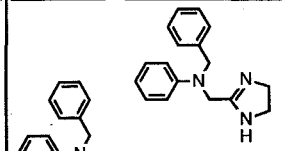
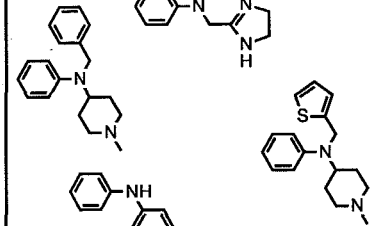
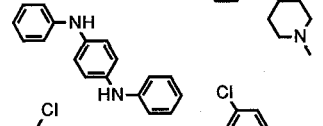
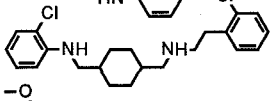
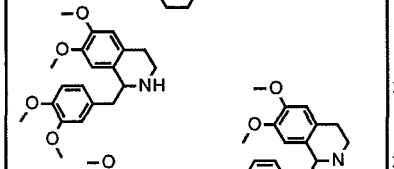
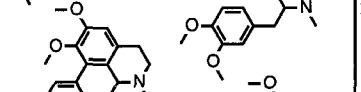
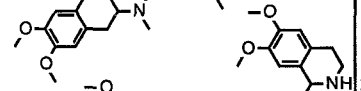
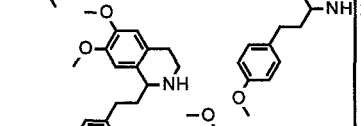
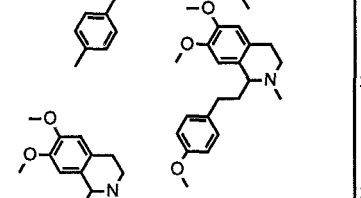
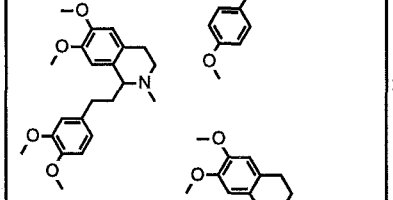
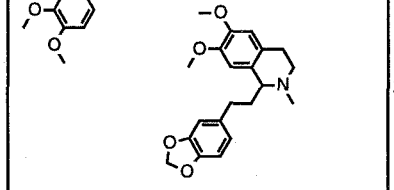
In the phenothiazines study the presence of a carbonyl group was found to enhance MDR-reversal activity; the question was therefore raised as to whether the presence of such a group would also affect the MDR-reversal activity of the bis(phenylalkyl)amines. As shown in Table 2, only 10.9% of the carbonyl-containing compounds presented in Table 1 produced an MDR-reversal ratio of <3, whereas 73.9% yielded a ratio of ≥ 10. These figures are similar to those found among the carbonyl-containing phenothiazines. It is also evident from Table 2 that among the carbonyl-containing compounds the presence of a tertiary amine group supports MDR-reversal activity to a greater extent than that of a secondary amine and that a cyclic amine function enhances the activity more than a noncyclic amine function. The same conclusions were drawn from the results obtained with the phenothiazine compounds, which points out their generality.

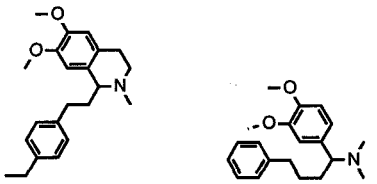
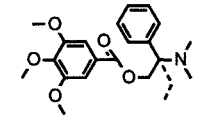
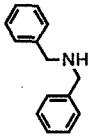
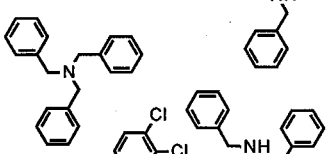
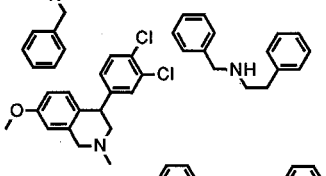
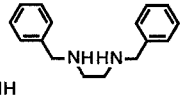
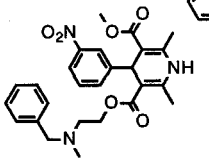
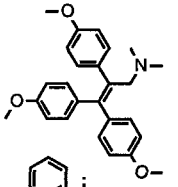
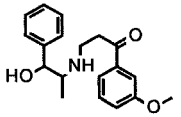
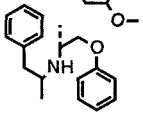
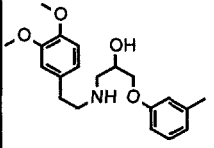
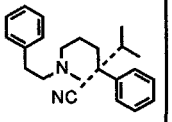
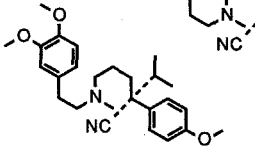
Among the carbonyl-lacking compounds, 21.7% produced an MDR-reversal ratio of <3, whereas 55.1% yielded a ratio of ≥ 10 (Table 2). This is a much higher proportion of MDR-reversal-active compounds than that found among the carbonyl-lacking phenothiazines. The comparison of the structure and activity of compounds 62 versus 54, 17 versus 15, and 59 and 60 versus 56 suggested that

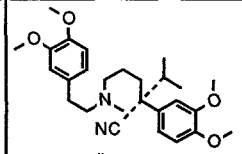
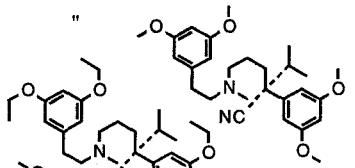
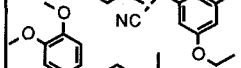
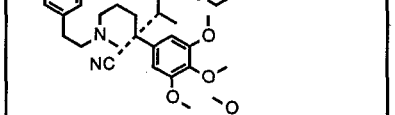
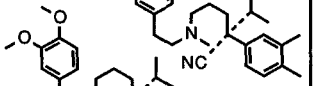
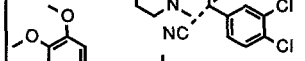
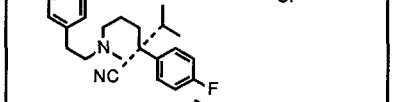
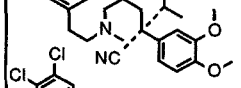
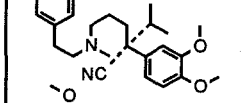
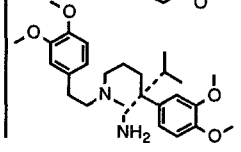
Table 1. Effectiveness of MDR reversal obtained using 115 compounds in P388 and P388/ADR cells in vitro

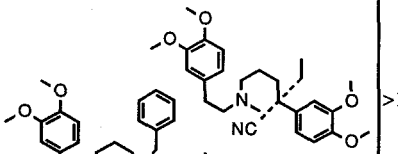
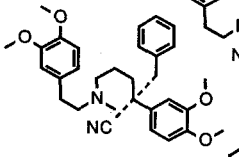
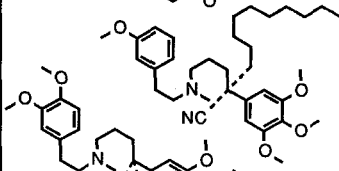
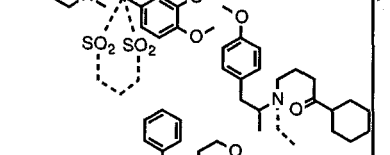
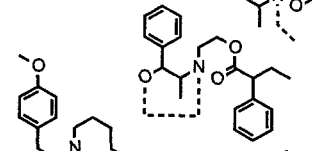
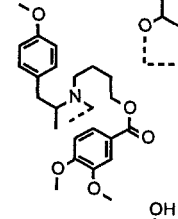
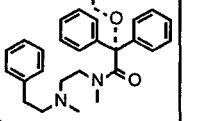
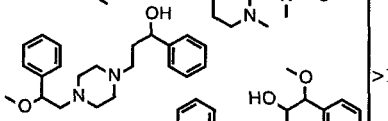
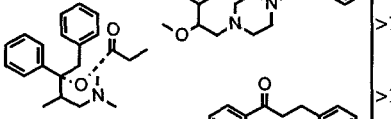
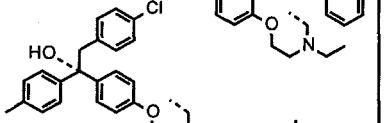
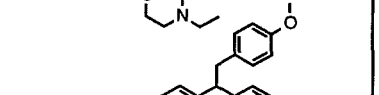
	COMPOUND		A	B	C
1	ethylbenzylaniline		>60	>60	1
2	Phenbenzamine		60	>100	>1.7
3	Histapyrrodine		80	80	2
4	Bepridil		3.5	11	4.2
5	Methaphenilene		80	80	1.6
6	MDL 6792		12	24	4.1
7	Fenoxedil		8	10	10
8	Terconazole R=  *= N		8	12	17.1
9	Ketoconazole R= 		20	20	16.7
10	R42,164 R= " *= N		>60	>60	>20
11	R41,300 R= 		8	10	22.2
12	R49,960 R= 		0.8	0.8	4
13	Itraconazole R= " *= N		0.8	0.8	4
14	Oxypertine		80	>100	>10
15	Milipertine		>100	>100	>10
16	Alpertine		45	20	33.3

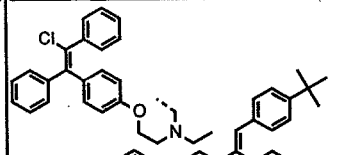
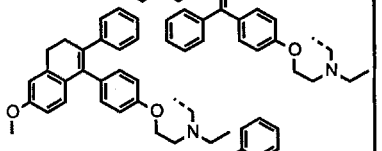
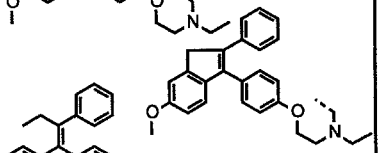
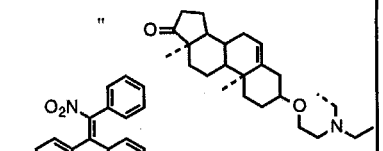
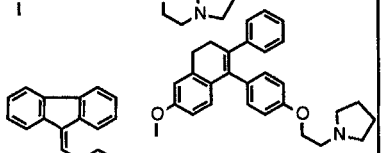
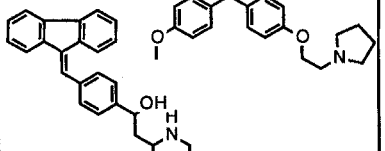
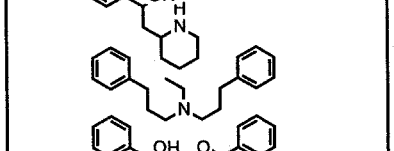
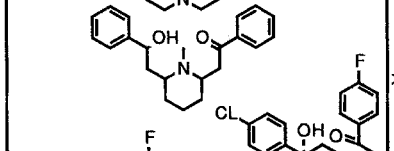
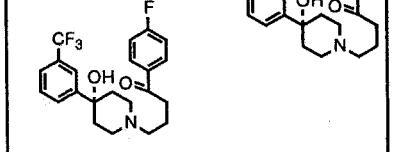
	COMPOUND		A	B	C
17	Solypertine		45	60	13.3
18	Enciprazine		>100	>100	>22.2
19	S 78 5781		>100	>100	>16.7
20	Selprazine		>100	>100	>16.7
21	Fluanisone		>60	>60	>7.5
22	Revenast		60	60	15
23	Peradoxime		>100	>100	>5
24	Tioperidone		12	12	20
25	Teroxalene		>60	>60	>12
26	Nefazodone		20	20	13.3
27	S79 0671		15	>60	>133.3

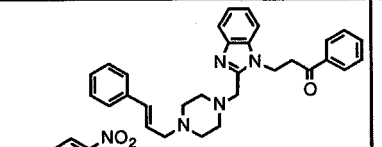
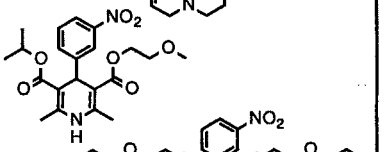
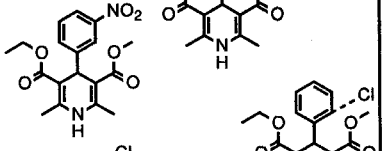
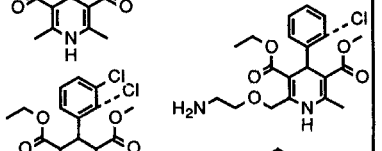
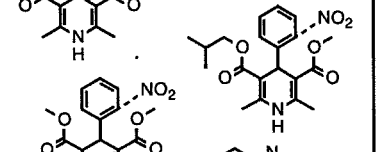
	COMPOUND		A	B	C
28	Antazoline		>100	>100	1
29	Bamipine		4.4	43	2
30	Thenaldine		60	80	1.3
31	N,N'-diphenyl-1,4-phenyl-diamine		15	>60	1
32	AY 9944		0.3	7.4	5.3
33	N-norlaudanosine		>100	>100	>20
34	Laudanosine		>600	>600	>40
35	Glaucine		80	80	80
36	Ro-04-2249		>100	>100	>100
37	Ro-04-2285		80	80	80
38	Ro-04-2250		>100	>100	>23
39	Ro-04-2359		>100	>100	>20
40	Ro-04-2360		>100	>100	>22

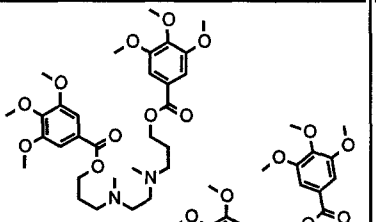
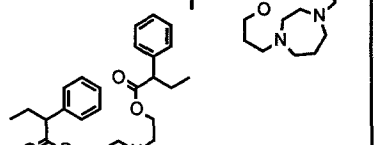
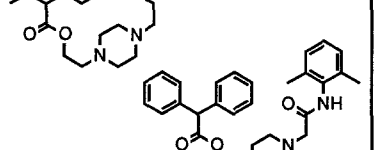
	COMPOUND		A	B	C
41	Ro-04-2669		20	60	50
42	Vetrabutine		80	80	10
43	Trimebutine		30	>100	>12.5
44	dibenzylamine		>60	>60	1
45	tribenzylamine		60	>100	>1.3
46	N-benzyl-2-phenethylamine		>60	>60	>3
47	Diclofensine		20	20	2.5
48	N,N'-dibenzylethylene diamine		>60	>60	1
49	Nicardipine		16	23	15.3
50	Amotriphene		1.6	20	5
51	Oxyfedrine		20	30	1
52	Racefemine		45	>60	>7.5
53	Bevantolol		>100	>100	>50
54	Emopamil		20	20	10
55	D557		45	20	44.4

	COMPOUND		A	B	C
56	Verapamil		80	80	100
57	(S)-Verapamil	"	80	80	100
58	(R)-Verapamil	"	80	80	100
59	D784		12	12	26.6
60	D894		8	8	40
61	Gallopamil		60	160	200
62	D559		20	20	100
63	D595		8	10	50
64	SZ48		20	20	25
65	T13		20	20	44.4
66	Devapamil		45	45	37.5
67	D792		8	8	17.8
68	D528		20	60	2

	COMPOUND		A	B	C
69	D525		>100	80	66.7
70	D490		20	20	100
71	Dagapamil		8	8	40
72	Tiapamil		>100	>100	>1.3
73	Secoverine		30	60	10
74	Fenbutrazate		30	45	10
75	Mebeverine		60	60	100
76	Carbiphene		1.5	8	10
77	Eprozinol		>100	>100	>5
78	Zipeprol		>100	>100	>5
79	Propoxyphene		>100	>100	>5
80	Etafenone		40	60	10
81	Triparanol		5	8	4.7
82	MER37		1.4	27	11.7

	COMPOUND		A	B	C
83	Clomiphene		3.5	4	2
84	MDL8917V		4.5	5.5	6.6
85	U10,520A		0.084	1.9	19
86	U11,555A		0.054	1.7	3.2
87	Tamoxifen		4.5	15	6
88	cis-Tamoxifen		3	5.5	3.9
89	U18,666A		4.5	12	60
90	Nitromifene		0.026	1.5	15
91	Nafoxidine		0.025	2.3	12.8
92	MDL10,393		5	8	5
93	Alverine		20	60	4
94	Lobeline		>100	>100	>6.6
95	Haloperidol		20	45	15
96	Trifluperidol		20	>30	>6.7

	COMPOUND		A	B	C
97	Cinprazole		20	15	18.8
98	Nimodipine		45	45	22.5
99	Niludipine		20	20	20
100	Nitrendipine		45	80	10
101	Amlodipine		13	20	10
102	Felodipine		20	30	2
103	Nisoldipine		20	20	2.5
104	Nifedipine		>30	>60	>1.4
105	Darodipine		45	50	11.1
106	Flordipine		>60	>60	>7.5
107	Lenperone		>60	>60	>13.3
108	Spirilene		>60	>60	50
109	Spiramide		60	60	20
110	Spasmadryl		>100	>100	>1
111	Azelastine		20	20	16.7

	COMPOUND		A	B	C
112	Hexobendine		100	>100	>125
113	Dilazep		20	50	50
114	Febuverine		60	60	50
115	SPS 1853		60	60	50

(*Column A* ED_{50} value in μM obtained in P388 cells, *column B* ED_{50} value in μM obtained in P388/ADR cells, *column C* ratio of the ED_{50} value obtained in P388/ADR cells in the absence of ADR to that determined in the presence of 0.2 μM ADR)

the replacement of a phenyl ring with a 3,4-dimethoxyphenyl or phenyldioxymethane or 3,5-dimethoxyphenyl or 3,5-diethoxyphenyl could augment the MDR-reversal activity in carbonyl-lacking compounds. As indicated in Table 2, among the carbonyl-lacking compounds that contained such groups, 93.9% produced an MDR-reversal ratio of ≥ 10 and 57.6% yielded a ratio of ≥ 30. These figures clearly indicate the enhancing effect of these residues on the MDR-reversal activity of the bis(phenylalkyl)amines. The role of such groups on the MDR-reversal activity of carbonyl-lacking phenothiazines is not known, as such compounds were not tested. Among the carbonyl-lacking compounds that did not contain dimethoxyphenyl groups, 36.1% produced an MDR-reversal ratio of <3 and 19.4% yielded a ratio of ≥ 10 (Table 2). These figures are similar to those obtained among the carbonyl-lacking phenothiazines.

Many of the verapamil-like compounds possessed a nitrile residue, which could have contributed to their high degree of MDR-reversal activity. However, the comparison of the structure and activity of compounds 54 versus 52 and 55 versus 53 indicates that the nitrile residue has no effect on MDR-reversal activity. The comparison of compounds 68 and 72 with compound 56 indicates that a primary amine or a 1,3-dithiane-1,1,3,3-tetraoxide group in the same location does ameliorate MDR-reversal activity.

In the phenothiazines study, it was shown that MDR-reversal activity could be maintained in compounds in which one or both phenyl rings were substituted by cyclopentyl, cyclohexyl, thienyl, or 5-norbornen-2-yl rings. In the present study the following ring structures were also found to support such activity: indole (compounds 14–17), 3,4-dihydrocarbostryl (compounds 19 and 20), quinazoline (compound 24), phthalazine (compound 111), and 1,3-benzodioxazole (compound 40).

In summary, the present and the previously reported phenothiazines studies suggest that MDR-reversal activity relates to the following structural features: two or three

Table 2. MDR-reversal activity of the compounds presented in Table 1 (grouped according to their structural features)

Structure	Number of compounds	MDR-reversal ratio				
		>3	≥3 but <5	≥5 but <10	≥10 but <30	≥30
With a carbonyl	46	5	2	5	25	9
Non-cyclic secondary amine	1	1				
Cyclic secondary amine	8	3			5	
Noncyclic tertiary amine	11	1		1	6	3
Cyclic tertiary amine	26		2	4	14	6
Without a carbonyl	69	15	7	9	19	19
+ Dimethoxyphenyl	33	2			12	19
– Dimethoxyphenyl	36	13	7	9	7	

phenyl rings (or other specific rings as indicated above) that are connected through one or two bridges (of a variety of types) to a cyclic or noncyclic secondary to tertiary amine group and that also possess carbonyl and/or dimethoxyphenyl functions.

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