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# Reversal of multidrug resistance by phenothiazines and structurally related compounds

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**Summary.** The multidrug-resistance (MDR)-reversal activity of 232 phenothiazines and structurally related compounds was tested in MDR P388 cells. Such activity was found among compounds exhibiting two ring structures (phenyl, cyclopentyl, cyclohexyl, thienyl or 5-norbornen-2-yl but not pyridinyl) linked by a variety of bridge types and possessing a secondary or tertiary amine group. Among 192 such compounds, 31.8% displayed good activity (MDR-reversal ratio, $\geqslant 10$) and 8.3%, outstanding activity (MDR-reversal ratio, $\geqslant 30$). In a subgroup comprising 56 compounds with a carbonyl residue, 4 with sulfuryl residue and 1 with thienyl residue, 42.7% showed good activity and 18%, outstanding activity. The contribution of these residues to the MDR-reversal activity was particularly evident among compounds containing a cyclic tertiary amine. Among 49 such compounds, 51% displayed good activity and 20.4%, outstanding activity, whereas among the 85 compounds lacking such groups, only 31.8% showed good activity and 4.7%, outstanding activity. Enhancement of this activity by the carbonyl group is also obtained when the latter is part of an amide bond of a tertiary amine. As compounds with a carbonyl group located on the rings, on the bridge to the amine group or beyond the amine are efficient MDR reversers, it seems that the exact molecular location of the carbonyl group is not critical for the elicitation of this activity.

## Introduction

Following the report of Tsuruo et al. in 1981 [23] on the in vitro reversal of multidrug resistance (MDR) by verapamil, a large number of compounds exhibiting diverse molecular structures and biological activities were reported to exert such activity (for references, see [2, 22]). Although the mechanisms by which these compounds restore drug sensitivity are poorly understood, efforts have been made to elucidate the structure-activity relationships among such compounds. Previous studies have been carried out using a rather limited number of compounds [6, 7, 11, 15, 27] or a group of analogues of an active agent such as verapamil [25], nicardipine [8, 10, 13, 26], trifluoperazine [3], flupenthixol [4], triparanol [15, 17], dipyridamole [15, 20], reserpine [14], cefoperazone [5] or cyclosporine [24]. These investigations revealed certain common structural features shared by many MDR reversers, but a definitively optimal structure indicative of such activity has not yet emerged. In a further attempt to elucidate the structure-activity relationship (SAR) of MDR chemosensitization, we present the results we obtained in vitro in P388/ADR cells using 232 phenothiazines and structurally related compounds.

## Materials and methods

The 232 compounds presented in Table 1 were generously donated by the following suppliers (listed by compound number): 132, Abbott Labs (Abbott Park, I11.); 99, Alfa Farmaceutici SpA (Bologna, Italy); 167, Labs Almirall SA (Barcelona, Spain); 162, Doctor Andreu SA (Barcelona, Spain); 185, Labs Andrommaco SA (Madrid, Spain); 32, 46 and 53, Asta Pharma AG (Frankfurt, FRG); 171, Labs A Bailly-SPEAB (Ivry/Seine, France); 10 and 201, Bristol-Myers Co. (Wallingford, Conn.); 217 and 218, BYK Gulden Pharmazeutika (Konstanz, FRG); 220, Chinoin Pharmaceutical & Chemical Works Ltd. (Budapest, Hungary); 56, 57, 104 and 138, Ciba-Geigy AG (Basle, Switzerland); 215, CTS Chemical Industries Ltd. (Petach Tikva, Israel); 100, Dainippon Pharmaceutical Co. (Osaka, Japan); 34 and 199, Egis Pharmaceuticals (Budapest, Hungary); 111, AB Ferrosan (Malmo, Sweden); 74, Fujisawa Pharmaceutical Co. (Osaka, Japan); 153, 155 and 156, Gist-Brocades Farmaca (Meppel, Holland); 189, Heumann Pharma GmbH (Feucht, FRG); 89, 90, 97, 135, 141–152, 202 and 203, Hoechst AG (Frankfurt, FRG); 136, F. Hoffmann La Roche (Basle, Switzerland); 196 and 198, Homburg (Frankfurt, FRG); 94, 107–110, 112–114, 120–122, 129, 130, 178, 208 and 209, Janssen Pharmaceutica (Beerse, Belgium); 88 and 93, Kabivitrum AB (Stockholm, Sweden); 172, KaliChemi AG (Hannover, FRG); 66 and 212, Knoll AG (Ludwigshafen, FRG); 60 and 61, Lederle (Wayne, N. J.); 33, 102 and 211, Lilly Research Labs (Indianapolis, Ind.); 75, 80, 81 and 134, H. Lundbeck A/S (Copenhagen-Valby, Denmark); 128, Lusopharmaco SpA (Milano, Italy); 98 and 165, Maggioni-Wintrop SpA (Milano, Italy); 103, 140, 159 and 160, McNeil

*Abbreviations:* ADR, Adriamycin; MDR, multidrug resistance; P388/ADR, multidrug-resistant P388 cells

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Pharmaceutical (Spring House, Pa.); 216, E. Merck (Darmstadt, FRG); 168, Merck Sharp & Dohme-Chibret AG (Glattbrugg, Switzerland); 20, 59, 62, 95, 139, 183, 184, 186, 192, 197 and 214, Merrell-Dow Pharmaceuticals (Cincinnati, Ohio); 222–232, Miles Inc. (West Haven, Conn.); 161, Montavit GmbH (Absam/Tirol, Austria); 157, 164 and 195, Parke-Davis (Ann Arbor, Mich.); 35, 78, 79, 119 and 123, Pfizer Central Research (Groton, Conn.); 101, Pharmuka Labs (Gennevilliers, France); 50, Pierrel SpA (Milano, Italy); 4, 6, 11, 15–17, 21, 22, 27, 38, 51, 82 and 124, Rhone-Poulenc Ltd. (Dagenham/Essex, UK); 18, 19, 25, 47, 106, 179 and 191, AH Robins Co. (Richmond, Va.); 219, Rowa Pharmaceuticals Ltd. (Bantry/Cork, Ireland); 13, 14, 26, 39, 58, 63–65, 67, 83, 85, 169 and 200, Sandoz Ltd. (Basle, Switzerland); 5 and 8, Sanofi Pharma (Paris, France); 29, 72, 91, 92 and 180, Schering-Plough Research (Bloomfield, N. J.); 131 and 204, Searle Research & Development (Skokie, I11.); 69, 190 and 205–207, Smith Kline & French Labs (King of Prussia, Pa.); 137, Sterling Drugs Inc. (Rensselaer, N. Y.); 12, 28, 31, 54 and 210, Taro Pharmaceutical Industries (Haifa Bay, Israel); 36, 117 and 154, Teva Pharmaceutical Industries (Petach Tikva, Israel); 96, Thiemann Arzneimittel (Waltrop, FRG); 52 and 70, Dr. K. Thomae (Biberach/Riss, FRG); 40–42, Prof. H. Timmerman (Amsterdam, Holland); 118, 119, 123, 125–127, 158, 170 and 173–177, UCB SA (Braine-l'Alleud, Belgium); 9, Upjohn Co. (Kalamazoo, Mich.); 193, UPSA Labs (Rueil-Malmaison, France); 182, Wellcome Foundation (London, UK); and 30, 37, 44 and 77, Wyeth-Ayerst Research (Princeton, N. J.). Compounds 1–3, 7, 23, 24, 43, 45, 48, 49, 55, 68, 71, 73, 76, 84, 86, 87, 105, 115, 116, 133, 163, 166, 181, 187, 188, 194, 213 and 221 were purchased from Sigma-Aldrich Israel (Petach Tikva, Israel).

Our standard test system has been described elsewhere [20]. Briefly, P388 murine leukemia cells and a doxorubicin-resistant subline (P388/ADR) were maintained in RPMI 1640 medium supplemented with 10% fetal calf serum, 10 μM 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: $1 \times 10^6$ cells were cultured in 10 ml medium in the presence of various drug concentrations. 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 curve 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 $ED_{50}$ values obtained in P388/ADR cells incubated in the absence versus the presence of 0.2 μM ADR; this ADR dose was just below the concentration that produced a detectable growth-inhibitory effect on these cells. (The ADR $ED_{50}$ values obtained in P388 and P388/ADR cells were $3.5 \times 10^{-8}$ M and $9 \times 10^{-7}$ 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 sub-inhibitory dose of the compound tested [16, 17]. Both experimental designs detected cytotoxic synergism between the tested compounds and ADR with equal efficiency. The real advantage of the experimental design used in the present 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

The results obtained for the 232 compounds tested 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 μM ADR) is shown in column C. Therefore, the values in column C represent the ability of the compounds to reverse MDR. The growth-inhibitory activity of almost every compound was also tested in drug-sensitive P388 cells in the presence of $1 \times 10^{-8}$ M ADR; however, in no case was a >2-fold decrease in the $ED_{50}$ observed (data not shown). In column D, the ratio between the $ED_{50}$ value obtained using each compound in P388/ADR cells and that determined using promazine (60 μM) in these cells was multiplied by the value in column C. The results serve as an index of MDR-reversal effectiveness in relation to that of promazine, a compound chosen as a standard for comparison [15].

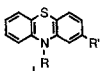
The comparison of MDR-reversal activity among compounds exhibiting such a variety of molecular structures (Table 1) could be analyzed in many ways, and numerous deductions could be made. In the following discussion, only certain conclusions are presented that seemed to us to be of major consequences to the SAR of this effect.

As shown in column C of the table, 77 compounds (33.2%) produced an MDR-reversal ratio of $\geq 10$ and 16 (6.9%) yielded a ratio of $\geq 30$. As is evident from the table, MDR-reversal activity can be obtained using many compounds that possess two phenyl rings linked by a thiazine (phenothiazines) or by one of a rather large variety of bridge types. Furthermore, as shown by the activities of compounds 67, 83–85, 139, 140, 184, 185 and 210–215, MDR-reversal activity is also obtained using compounds in which one or even both phenyl rings have been substituted by cyclopentyl, cyclohexyl, thienyl or 5-norbornen-2-yl rings. However, of the 18 compounds in which a pyridine substituted for one of the phenyl rings, only 3 produced an MDR-reversal ratio of $\geq 3$ and none yielded a ratio of $\geq 10$. Therefore, it is clear that this type of ring structure does not support MDR-reversal activity. Comparisons of the activity of compounds 115 and 116 to that of compound 133 and the activity of compound 144 to that of compounds 145–148 suggest that drugs exhibiting a single ring are less active than those possessing two rings.

It has previously been suggested that the MDR-reversal activity of compounds containing secondary or tertiary amine residues is stronger than that of compounds possessing other amine groups and that compounds with a piperidine or a piperazine group exert greater activity than do those with a non-cyclic amine moiety [2, 15]. Of the compounds tested in the present study, 13 either lacked a secondary or tertiary amine group or contained such an amine as a carboxamide. No MDR-reversal activity was obtained using these compounds, except for two that produced an MDR-reversal ratio of <3. Therefore, the following discussion is limited to 192 compounds exhibiting a two-linked-ring structure (excluding pyridine) and a secondary or tertiary amine group.

Ford et al. [3] have shown that certain substitutions in position 2 of the phenothiazine ring enhance MDR-reversal activity. The order of activity shown by the substituents was $CF_3$>Cl>$SCH_3$>H; however, the increments in activity were rather small. The activities of compounds 1–5, 7, 8, 12–14, 22–27 and 28–31 indicate that although substi-

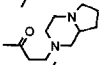
**Table 1.** Effectiveness of MDR reversal obtained using 232 phenothiazines and structurally related compounds in P388 and P388/ADR cells in vitro



| | COMPOUND | R' | R | A | B | C | D |
|---|---|---|---|---|---|---|---|
| 1 | promazine | |  | 40 | 60 | 3 | 3 |
| 2 | chlorpromazine | Cl | " | 12 | 20 | 2.5 | 0.8 |
| 3 | triflupromazine | $CF_3$ | " | 12 | 20 | 2.5 | 0.8 |
| 4 | methopromazine | $OCH_3$ | " | 20 | 20 | 4.4 | 1.5 |
| 5 | acepromazine | $COCH_3$ | " | 30 | 45 | 5.6 | 4.2 |
| 6 | trimeprazine | |  | 20 | 20 | 2 | 0.7 |
| 7 | promethazine | |  | 60 | 60 | 7.5 | 7.5 |
| 8 | aceprometazine | $COCH_3$ | " | 40 | 80 | 6 | 8 |
| 9 | pyrathiazine | |  | 60 | 60 | 5 | 5 |
| 10 | methdilazine | |  | 20 | 20 | 2.5 | 0.8 |
| 11 | mequitazine | |  | 12 | 12 | 2.7 | 0.5 |
| 12 | thioridazine | $SCH_3$ |  | 8 | 12 | 6 | 1.2 |
| 13 | mesoridazine | $SOCH_3$ | " | 40 | 60 | 13.3 | 13.3 |
| 14 | sulforidazine | $SO_2CH_3$ | " | 20 | 20 | 10 | 3.3 |
| 15 | perimetazine | $OCH_3$ |  | 10 | 20 | 10 | 3.3 |
| 16 | propericiazine | CN |  | 20 | 20 | 1 | - |
| 17 | metopimazine | $SO_2CH_3$ |  | >60 | >60 | 1 | - |
| 18 | duoperone | $CF_3$ |  | 10 | 12 | 26.7 | 5.3 |
| 19 | AHR 06601 | $COCH_3$ |  | 10 | 12 | 20 | 4 |
| 20 | piperacetazine | $COCH_3$ |  | 20 | 20 | 4.4 | 1.5 |
| 21 | pipotiazine-palmitate | $SO_2N(CH_3)_2$ |  | >100 | >100 | > 1 | > 1.7 |

| | COMPOUND | $R_1$ | $R_2$ | R | A | B | C | D |
|---|---|---|---|---|---|---|---|---|
| 39 | methixene | CH | S |  | 10 | 12 | 2.7 | 0.5 |
| 40 | hepzidine | CH | $CH_2CH_2$ |  | 60 | >100 | 12 | >20 |
| 41 | tropirine | N | CHCH |  | 60 | >100 | 8 | >13.3 |
| 42 | tixadil | CH | S |  | 4.5 | 4.5 | 3.8 | 0.3 |
| 43 | Aldrich 21430-2 | CH | $CH_2CH_2$ |  | 60 | 60 | 45 | 45 |
| 44 | citenamide | CH | CHCH |  | >60 | >60 | 1 | - |
| 45 | desipramine | CH | $CH_2CH_2$ |  | 40 | 60 | 3 | 3 |
| 46 | prothipendyl | N | S |  | 60 | 60 | 1.3 | 1.3 |
| 47 | tampramine | N | CN | " | 60 | 60 | 7.5 | 7.5 |
| 48 | imipramine | CH | $CH_2CH_2$ | " | 60 | 60 | 3 | 3 |
| 49 | trimipramine | CH | $CH_2CH_2$ |  | 60 | 60 | 6 | 6 |
| 50 | azipramine | C | $CH_2CH_2$ |  | 20 | 45 | 37.5 | 28.1 |
| 51 | quinupramine | CH | $CH_2CH_2$ |  | 60 | 60 | 8 | |
| 52 | pirenzepine | N | CNH |  | >60 | >60 | 1 | - |
| 53 | oxypendyl | N | S |  | 60 | 60 | 2 | 2 |
| 54 | opipramol | CH | CHCH | " | 60 | 60 | 3 | 3 |



| | COMPOUND | R' | R | A | B | C | D |
|---|---|---|---|---|---|---|---|
| 22 | perazine | |  | 12 | 20 | 4.4 | 1.5 |
| 23 | prochlorperazine | Cl | " | 4.5 | 8 | 6.7 | 0.9 |
| 24 | trifluoperazine | $CF_3$ | " | 4.5 | 8 | 10 | 1.3 |
| 25 | butaperazine | $COCH_2CH_2CH_3$ | " | 4.5 | 8 | 17.8 | 2.4 |
| 26 | thiethylperazine | $SCH_2CH_3$ | " | 4.5 | 8 | 10 | 1.3 |
| 27 | thioproperazine | $SO_2N(CH_3)_2$ | " | 8 | 20 | 16.7 | 5.6 |
| 28 | perfenazine | Cl |  | 8 | 10 | 5 | 0.8 |
| 29 | acetophenazine | $COCH_3$ | " | 15 | 20 | 10 | 3.3 |
| 30 | carphenazine | $COCH_2CH_3$ | " | 12 | 12 | 15 | 3 |
| 31 | fluphenazine | $CF_3$ | " | 8 | 8 | 4 | 0.5 |
| 32 | homofenazine | $CF_3$ |  | 2 | 6 | 4 | 0.4 |
| 33 | cyclofenazine | $CF_3$ |  | 20 | 20 | 16.7 | 5.6 |
| 34 | metofenazate | Cl |  | 3 | 6 | 20 | 2 |
| 35 | imiclopazine | Cl |  | 200 | 200 | 1.7 | 5.7 |
| 36 | dixyrazine | |  | 20 | 20 | 10 | 3.3 |
| 37 | azaclorazine | Cl |  | 40 | 40 | 10 | 6.7 |
| 38 | chloracyzine | Cl |  | 60 | 60 | 5 | 5 |

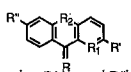


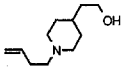
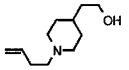
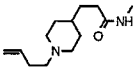
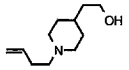
| | COMPOUND | R' | $R_2$ | R | A | B | C | D |
|---|---|---|---|---|---|---|---|---|
| 55 | mianserin | | $CH_2$ |  | 60 | 60 | 3 | 3 |
| 56 | maroxepine | | O |  | 60 | 60 | 3 | 3 |
| 57 | citatepine | CN | S | " | 20 | 60 | 13.3 | 13.3 |

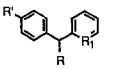


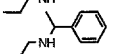
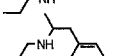
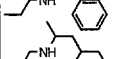
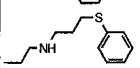
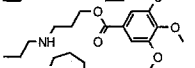
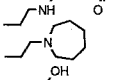
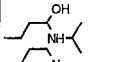
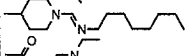
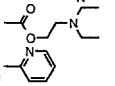
| | COMPOUND | R' | R" | $R_2$ | | A | B | C | D |
|---|---|---|---|---|---|---|---|---|---|
| 58 | clothiapine | Cl | | S | | 45 | 60 | 13.3 | 13.3 |
| 59 | metiapine | $CH_3$ | | S | | 60 | 60 | 13.3 | 13.3 |
| 60 | loxapine | Cl | | O | | 60 | 60 | 13.3 | 13.3 |
| 61 | amoxapine | Cl | | O | **desmethyl | 15 | 60 | 13.3 | 13.3 |
| 62 | metoxepin | $OCH_3$ | | O | | 20 | 45 | 5.6 | 4.2 |
| 63 | clozapine | | Cl | NH | | >100 | >100 | >3.3 | >5.5 |
| 64 | perlapine | | | $CH_2$ | | >100 | >100 | >5 | >8.3 |
| 65 | fluperlapine | | F | $CH_2$ | | 60 | 60 | 5 | 5 |
| 66 | rilapine | | Cl | >CCHCN | *CH not N | >60 | >60 | >40 | >40 |
| 67 | tilozepine | | |  | | 20 | 60 | 10 | 10 |

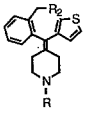
**Table 1** (continuation)

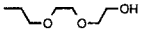


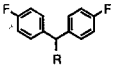
| | COMPOUND | $R_1$ | $R_2$ | R' | R'' | R | A | B | C | D |
|---|---|---|---|---|---|---|---|---|---|---|
| 68 | pimethixene | CH | S | | |  | 20 | 45 | 5.6 | 4.2 |
| 69 | clopipazan | " | O | Cl | " | " | 18 | 25 | 5.6 | 2.3 |
| 70 | danitracen | " |  | | | " | 20 | 20 | 1 | - |
| 71 | cyproheptadine | " | CHCH | | | " | 40 | 50 | 5 | 4.2 |
| 72 | azatadine | N | $CH_2CH_2$ | | | " | >100 | >100 | >1.3 | >2.2 |
| 73 | amitriptylin | CH | " | | |  | 45 | 60 | 5 | 5 |
| 74 | piroheptin | " | " | | |  | 60 | 60 | 13.3 | 13.3 |
| 75 | piflutixol | " | S | $CF_3$ | F |  | 8 | 12 | 26.7 | 5.3 |
| 76 | cis-thiothixene | " | S | $SO_2N(CH_3)_2$ | |  | 12 | 20 | 25 | 8.3 |
| 77 | clopenthixol | " | S | Cl | |  | 10 | 12 | 15 | 3 |
| 78 | clothixamide | " | S | Cl | |  | 20 | 20 | 20 | 6.7 |
| 79 | pinoxepine | " | $CH_2O$ | Cl | |  | 12 | 15 | 12.5 | 3.1 |
| 80 | cis-flupentixol | " | S | $CF_3$ | | " | 8 | 12 | 15 | 3 |
| 81 | trans- " | " | S | $CF_3$ | | " | 4.5 | 8 | 10 | 1.3 |
| 82 | nuclotixene | " | S | Cl | |  | 8 | 8 | 1.2 | 0.2 |



| | COMPOUND | $R_1$ | R' | R | A | B | C | D |
|---|---|---|---|---|---|---|---|---|
| 86 | 2,2'-diphenylethylamine | CH | |  | >100 | >100 | 1 | - |
| 87 | diphenamid | CH | |  | >60 | >60 | 1 | - |
| 88 | recipaverin | CH | |  | >100 | >100 | >1.7 | >2.8 |
| 89 | tolpropamine | CH | $CH_3$ |  | 80 | 80 | 4 | 5.3 |
| 90 | pheniramine | N | | " | >100 | >100 | 1 | - |
| 91 | chlorpheniramine | N | Cl | " | >100 | >100 | >1 | >1.7 |
| 92 | brompheniramine | N | Br | " | >100 | >100 | >1.7 | >2.8 |
| 93 | emepronium | CH | |  | >100 | >100 | 1 | - |
| 94 | diisopromine | CH | |  | 60 | 60 | 4 | 4 |
| 95 | terodiline | CH | |  | 60 | 60 | 6 | 6 |
| 96 | fendiline | CH | |  | 3 | 6 | 10 | 1 |
| 97 | prenylamine | CH | |  | 8 | 8 | 10 | 1.3 |
| 98 | droprenylamine | CH | |  | 8 | 8 | 10 | 1.3 |
| 99 | tiopropamine | CH | |  | 8 | 12 | 4 | 0.8 |
| 100 | mepramidil | CH | |  | 6 | 10 | 50 | 8.3 |
| 101 | prozapine | CH | |  | 20 | 45 | 4.5 | 3.4 |
| 102 | drobuline | CH | |  | >100 | >100 | > 5 | > 8.3 |
| 103 | fenoctimine | CH | |  | 6 | 8 | 1.3 | 0.2 |
| 104 | adiphenine | CH | |  | >100 | >100 | 1 | - |
| 105 | diphenyl-2-pyridylmethane | CH | |  | 80 | >100 | >2.2 | >3.7 |



| | COMPOUND | $R_2$ | R | A | B | C | D |
|---|---|---|---|---|---|---|---|
| 83 | pizotyline | $CH_2$ | $-CH_3$ | 20 | 45 | 5.6 | 4.2 |
| 84 | ketotifene |  | $-CH_3$ | >100 | >100 | >3.3 | >5.5 |
| 85 | etolotifene |  |  | >100 | >100 | >22.2 | >37.7 |



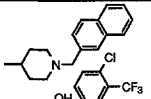
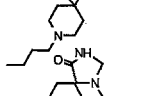
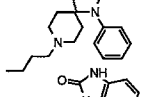
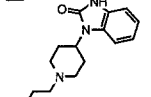
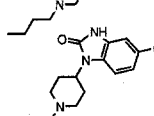
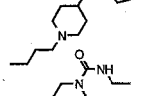
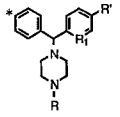
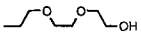
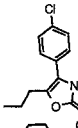
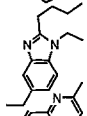
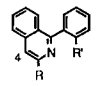
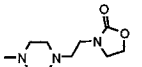
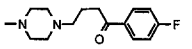
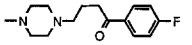
| | COMPOUND | R | A | B | C | D |
|---|---|---|---|---|---|---|
| 106 | AHR 16462 |  | 20 | 20 | 13.3 | 4.3 |
| 107 | penfluridol |  | 1.3 | 3 | 6.7 | 0.3 |
| 108 | fluspirilene |  | 8 | 8 | 13.3 | 1.8 |
| 109 | pimozide |  | 4.5 | 8 | 40 | 5.3 |
| 110 | clopimozide |  | 4.5 | 6 | 30 | 3 |
| 111 | amperozide |  | 20 | 50 | 6.3 | 5.2 |
| 112 | lidoflazine |  | 4.7 | 10 | 7.1 | 1.2 |
| 113 | mioflazine |  | 4.5 | 12 | 12 | 2.4 |
| 114 | difluanine |  | 8 | 8 | 17.8 | 2.4 |

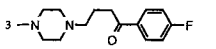
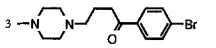
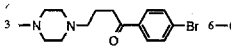
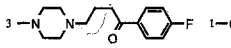
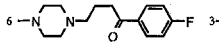
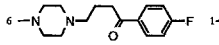
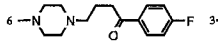
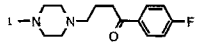
**Table 1** (continuation)

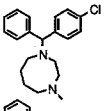
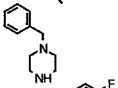
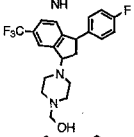
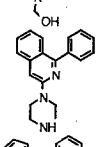
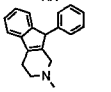
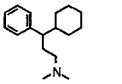
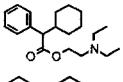
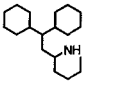
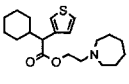


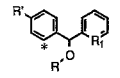
| | COMPOUND | $R_1$ | R' | R | A | B | C | D |
|---|---|---|---|---|---|---|---|---|
| 115 | 1-(4-chlorobenz-hydryl)piperazine | CH | Cl | H | 13 | 20 | 2.5 | 0.8 |
| 116 | cyclizine | CH | | $-CH_3$ | >100 | >100 | > 2 | > 3.3 |
| 117 | chlorocyclizine | CH | Cl | $-CH_3$ | 5.7 | 21 | 2.3 | 0.8 |
| 118 | meclizine | CH | Cl |  | 1.4 | 27 | 11.7 | 5.3 |
| 119 | buclizine | CH | Cl |  | 20 | 60 | 6 | 6 |
| 120 | clocinizine | CH | Cl |  | 20 | 60 | 60 | 60 |
| 121 | cinnarizine | CH | | " | 27 | 17 | 6.8 | 1.9 |
| 122 | flunarizine | CH | F | " *—F | 12 | 19 | 5.6 | 1.8 |
| 123 | hydroxyzine | CH | Cl |  | 60 | 60 | 7.5 | 7.5 |
| 124 | piclopastine | N | | " *—Cl | >100 | >100 | >1.7 | > 2.8 |
| 125 | UCB-L172 | CH | Cl |  | 60 | 60 | 10 | 10 |
| 126 | cetrizine | CH | Cl |  | >100 | >100 | 1 | - |
| 127 | etodroxizine | CH | Cl |  | 30 | 20 | 4.4 | 1.5 |
| 128 | LR-A/028 | CH | Cl |  | 15 | 20 | 4 | 1.3 |
| 129 | oxatomide | CH | |  | 12 | 15 | 10 | 2.5 |
| 130 | buterizine | CH | |  | 8 | 13 | 65 | 14.1 |
| 131 | ropizine | CH | |  | 45 | 30 | 5 | 2.5 |



| | COMPOUND | R' | R | A | B | C | D |
|---|---|---|---|---|---|---|---|
| 141 | S79 1099 | |  | 8 | 8 | 8 | 1.1 |
| 142 | S79 1100 | |  | 20 | 20 | 10 | 3.3 |
| 143 | S79 0491 | OH |  | 20 | 45 | 22.5 | 16.9 |
| 144 | S79 0671 | $OCH_3$ |  | 15 | >60 | >133.3 | >133.3 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 145 | cinuperone |  | >100 | >100 | >8.3 | >13.3 |
| 146 | S81 0339 |  | > 60 | > 60 | > 13.3 | >13.3 |
| 147 | S81 2521 |  | > 60 | > 60 | > 2 | >2 |
| 148 | S79 2823 |  | 20 | 30 | 15 | 7.5 |
| 149 | S83 4163 |  | 12 | 12 | 10 | 2 |
| 150 | S83 4096 |  | 4.5 | 4.5 | 5.6 | 0.4 |
| 151 | S81 0337 |  | 20 | 20 | 10 | 3.3 |
| 152 | S81 1951 |  | 45 | 30 | 6.7 | 3.3 |

| | COMPOUND | | A | B | C | D |
|---|---|---|---|---|---|---|
| 132 | homochlorocyclizine |  | 10 | 20 | 2.5 | 0.8 |
| 133 | 1-benzylpiperazine |  | >100 | >100 | 1 | - |
| 134 | tefludazine |  | 15 | 20 | 10 | 3.3 |
| 135 | perafensine |  | 12 | 20 | 3.3 | 1.1 |
| 136 | phenindamine |  | >100 | >100 | >2.2 | >3.7 |
| 137 | gamfexine |  | 60 | 60 | 1 | - |
| 138 | drofenine |  | >100 | >100 | 1 | - |
| 139 | perhexiline |  | 7 | 15 | 3 | 0.8 |
| 140 | cetiedil |  | 4.5 | 20 | 10 | 3.3 |



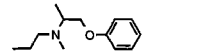
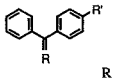
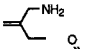
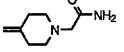
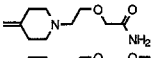
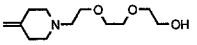
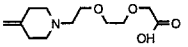
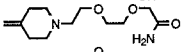
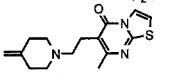
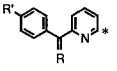
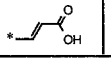
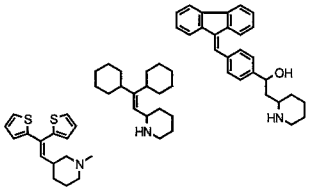
| | COMPOUND | $R_1$ | R' | R | A | B | C | D |
|---|---|---|---|---|---|---|---|---|
| 153 | tofenacin | CH | |  | 60 | >100 | >1.7 | >2.8 |
| 154 | diphenhydramine | CH | |  | >100 | >100 | >1.7 | >2.8 |
| 155 | orphenadrine | CH | | " *—$CH_3$ | > 60 | > 60 | >1.3 | >1.3 |
| 156 | neobenodine | CH | $CH_3$ | " | >100 | >100 | >1.7 | >2.8 |
| 157 | ambodryl | CH | Br | " | 8 | 30 | 3 | 1.5 |
| 158 | medrylamine | CH | $OCH_3$ | " | > 60 | > 60 | >1 | >1 |
| 159 | carbinoxamine | N | Cl | " | >100 | >100 | >1 | >1.7 |
| 160 | (-) rotoxamine | N | Cl | " | >100 | >100 | >1 | >1.7 |
| 161 | etanautine | CH | |  | 60 | >100 | >1.7 | >2.8 |
| 162 | prenoverine | CH | |  | 45 | 45 | 3.8 | 2.9 |
| 163 | cloperastine | CH | Cl |  | 2 | 60 | 10 | 10 |
| 164 | linadryl | CH | |  | 60 | >100 | >5 | >8.3 |
| 165 | diphenazoline | CH | |  | >100 | >100 | 1 | - |
| 166 | diphenylpyraline | CH | |  | 60 | >100 | >5 | >8.3 |
| 167 | ebastine | CH | |  | 4.5 | 4.5 | 10 | 0.8 |
| 168 | benztropine | CH | |  | 8 | 60 | 10 | 10 |
| 169 | ethylbenztropine | CH | |  | 30 | 60 | 10 | 10 |
| 170 | chlorbenzoxamine | CH | |  | 8 | 20 | 25 | 8.3 |
| 171 | captodiame | | |  | 4.5 | 8 | 4 | 5.3 |

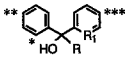
**Table 1** (continuation)

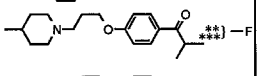
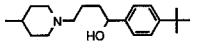
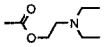


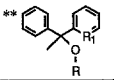
| | COMPOUND | R' | R | A | B | C | D |
|---|---|---|---|---|---|---|---|
| 172 | etifelmine | |  | 60 | 60 | 3 | 3 |
| 173 | UCB-26166 | Cl |  | 80 | 80 | 6.7 | 8.9 |
| 174 | UCB-26359 | $OCH_3$ |  | 80 | 80 | 10 | 13.3 |
| 175 | pipoxizine | |  | 60 | 80 | 10 | 13.3 |
| 176 | UCB-J028 | |  | >100 | >100 | 1 | - |
| 177 | UCB-N101 | |  | 60 | 60 | 13.3 | 13.3 |
| 178 | R59022 | F |  | 35 | 45 | 4.5 | 3.4 |
| 179 | pridefine | |  | 60 | >100 | > 5 | > 8.3 |



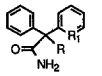
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 180 | cycliramine | Cl |  | 80 | >100 | > 1 | > 1.7 |
| 181 | triprolidine | $CH_3$ |  | >100 | >100 | > 2.2 | > 3.7 |
| 182 | acrivastine | $CH_3$ | "  | >60 | >60 | 1 | - |
| 183 | MDL-10393 | |  | 5 | 8 | 4.4 | 0.6 |
| 184 | hexadiline | | | 4.5 | 8 | 2.7 | 0.4 |
| 185 | tipepidine | | | 45 | >60 | >3 | >3 |

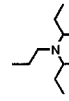


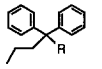
| | COMPOUND | $R_1$ | R | A | B | C | D |
|---|---|---|---|---|---|---|---|
| 186 | pipradol | CH |  | >100 | >100 | >1.7 | >2.8 |
| 187 | chlophedianol | CH |  *—Cl | >100 | >100 | 1 | - |
| 188 | pridinol | CH |  | >60 | >60 | >2 | >2 |
| 189 | butinoline | CH |  | >100 | >100 | >1.7 | >2.8 |
| 190 | diphenidol | CH |  | >100 | >100 | >5 | >8.3 |
| 191 | AHR-16303 | CH |  | 4.5 | 4.5 | 10 | 0.8 |
| 192 | terfenadine | CH |  | 2 | 4.5 | 5.6 | 0.4 |
| 193 | difemerine | CH |  | >100 | >100 | >1.7 | >2.8 |
| 194 | benactyzine | CH |  | >100 | >100 | 1 | - |
| 195 | pirmenol | N |  | >100 | >100 | >3.3 | >5.5 |



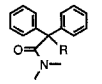
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 196 | chlorphenoxamine | CH |  **—Cl | 50 | 50 | 4.2 | 3.5 |
| 197 | doxylamine | N | " | >100 | >100 | > 1 | > 1.7 |
| 198 | mecloxamine | CH |  **—Cl | 20 | 25 | 3.1 | 1.3 |
| 199 | setastine | CH |  **—Cl | 12 | 12 | 6 | 1.2 |
| 200 | clemastine | CH |  **—Cl | 8 | 20 | 2.5 | 0.8 |



| | COMPOUND | $R_1$ | R | A | B | C | D |
|---|---|---|---|---|---|---|---|
| 201 | aminopentamide | CH |  | >100 | >100 | 1 | - |
| 202 | fenpipramide | CH |  | >100 | >100 | 1 | - |
| 203 | fenpiverinium | CH |  | >100 | >100 | 1 | - |
| 204 | disopyramide | N |  | >100 | >100 | 1 | - |



| | | | | | | |
|---|---|---|---|---|---|---|
| 205 | proadifen |  | 10 | 20 | 5 | 1.7 |
| 206 | SKF-3301 |  | 20 | 20 | 4.4 | 1.5 |
| 207 | SKF-16467 |  | 60 | >100 | >5 | >8.3 |

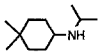
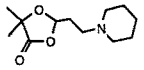
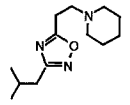


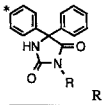
| | | | | | | |
|---|---|---|---|---|---|---|
| 208 | loperamide |  | 13 | 20 | 44.4 | 14.8 |
| 209 | fluperamide |  | 8 | 8 | 40 | 5.3 |

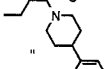
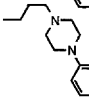


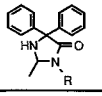
| | COMPOUND | $R_{11}$ | R | A | B | C | D |
|---|---|---|---|---|---|---|---|
| 210 | trihexylphenidyl |  |  | 60 | 60 | 3 | 3 |
| 211 | cycrimine |  | " | >100 | >100 | >2.2 | >3.7 |
| 212 | biperiden |  | " | >60 | >60 | >2 | >2 |
| 213 | procyclidine |  |  | >100 | >100 | >2.2 | >3.7 |
| 214 | propenzolate |  |  | 60 | 60 | 7.5 | 7.5 |
| 215 | oxybutynin |  |  | 60 | 60 | 13.3 | 13.3 |

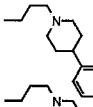
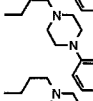
**Table 1** (continuation)

| | | | | | | |
|---|---|---|---|---|---|---|
| 216 | pramiverine |  | 20 | 35 | 3 | 1.8 |
| 217 | prodipine |  | 80 | 80 | 4 | 5.3 |
| 218 | budipine |  | 60 | 80 | 4 | 5.3 |
| 219 | pipoxolan |  | 8 | 12 | 1.5 | 0.3 |
| 220 | prenoxdiazine |  | 60 | 60 | 20 | 20 |



| COMPOUND | R | A | B | C | D |
|---|---|---|---|---|---|
| 221 phenytoin | H | 12 | >100 | 1 | - |
| 222 MA 1862 |  | >100 | >100 | 1 | - |
| 223 MA 1865 |  | >100 | >100 | 1 | - |
| 224 MA 1813 |  | >60 | >60 | 1 | - |
| 225 MA 1588 |  | >60 | >60 | >7.5 | >7.5 |
| 226 MA 1598 |  | 20 | 20 | 66.7 | 22.2 |
| 227 ropitoin | "  | 12 | 20 | 44.4 | 14.8 |
| 228 MA 1586 |  | 20 | 20 | 44.4 | 14.8 |



| | | | | | |
|---|---|---|---|---|---|
| 229 MA 1880 |  | >100 | >100 | 1 | - |
| 230 MA 1510 |  | 20 | 20 | 25 | 8.3 |
| 231 MA 1509 |  | 45 | >60 | >50 | >50 |
| 232 MA 1499 |  | 20 | 45 | 56.3 | 42.2 |

Column A, $ED_{50}$ values (μM) obtained in P388 cells; column B, $ED_{50}$ values 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 its presence (0.2 μM); column D, The ratio of the $ED_{50}$ value obtained in P388/ADR cells using the experimental compound(s) to that determined using 60 μM promazine multiplied by the value in column C

tution in position 2 of the phenothiazine ring with Cl, $CF_3$, $OCH_3$, $SCH_3$ or $SCH_2CH_3$ indeed somewhat improved the MDR-reversal activity, a much stronger enhancement of activity was obtained by substitution with $COCH_3$, $COCH_2CH_3$, $COCH_2CH_2CH_3$, $SOCH_3$, $SO_2CH_3$ or $SO_2N(CH_3)_2$.

The MDR-reversal activity obtained using compounds 34 and 37 suggested that an augmentation of the activity could also result from a carbonyl substitution at locations other than position 2 of the phenothiazine structure. Of 37 phenothiazine compounds tested (compound 21, a palmitate ester devoid of activity, was excluded), 16 contained a carbonyl (or thionyl or sulfuryl) residue; 10 compounds (62.5%) of this sub-group produced an MDR-reversal ratio of $\geqslant 10$ as compared with 5 compounds (23.8%) among the carbonyl-lacking phenothiazines. It therefore seems that a carbonyl substitution, whether on the rings, on the bridge to the amine group or at a location beyond the amine, enhances the MDR-reversal activity of phenothiazines.

To determine whether the enhancement of MDR-reversal activity obtained by carbonyl substitution occurs only among phenothiazines or whether it is a general phenomenon, we investigated the MDR-reversal activity of all 192 compounds under discussion in relation to the presence or absence of such a residue. Among the group comprising 56 carbonyl-, 4 sulfuryl- and thienyl-substituted compounds (2 carboxyl-containing drugs, compounds 126 and 176, which are not active, were excluded), 37 compounds (60.7%) produced an MDR-reversal ratio of $\geqslant 10$ as compared with 36 (28.1%) of the compounds lacking a carbonyl group and 11 (18%) yielded a ratio of $\geqslant 30$ as compared with 5 (3.9%) carbonyl-lacking compounds. We therefore suggest that carbonyl substitution enhances MDR-reversal activity not in phenothiazine compounds alone, but rather in a wider range of compounds exhibiting a two-linked-ring structure that is connected to a secondary or tertiary amine.

Among the 128 carbonyl-lacking compounds under discussion, 17 contained a secondary amine group, and of these, only 4 (23.5%) produced an MDR-reversal ratio of $\geqslant 10$ (1 of 7 cyclic and 3 of 10 non-cyclic drugs) and none produced a ratio of $\geqslant 30$. Among the 111 tertiary-amine-containing compounds that possessed no carbonyl group,

32 (28.8%) produced a ratio of ⩾10 and 5 (4.5%), a ratio of ⩾30. This result suggests that in the group of carbonyl-lacking compounds, those with tertiary amines are only marginally more active than those with secondary amines. Among the 37 carbonyl-lacking piperidine compounds, 13 (35.1%) produced an MDR-reversal ratio of ⩾10 and none produced a ratio of ⩾30, whereas among the 33 corresponding piperazines, 15 (45.5%) compounds yielded a ratio of ⩾10 and 3 (9.1%), a ratio of ⩾30. It is therefore clear that the MDR-reversal activity of carbonyl-lacking, cyclic tertiary amine compounds is greater than that of the corresponding non-cyclic tertiary amine compounds (25 of 26 such compounds produced an MDR-reversal ratio of <10). However, the activity of even the most potent subgroup of these cyclic tertiary amine compounds, the piperazines, was considerably inferior to that of carbonyl-containing compounds. As indicated above, only 5 of the carbonyl-lacking drugs (compounds 43, 50, 66, 120 and 130) yielded an MDR-reversal ratio of ⩾30.

Only one carbonyl-containing drug with a secondary amine group (compound 100) was tested. This non-cyclic amine compound exerted strong MDR-reversal activity (50-fold), whereas the carbonyl-lacking, secondary amine compounds (cyclic or non-cyclic) exhibited low MDR-reversal activity, as shown above.

Among the 60 carbonyl-containing compounds possessing tertiary amine groups, 36 (60%) produced an MDR-reversal ratio of ⩾10, and of these, 10 (16.7%) yielded a ratio of ⩾30. Among the 27 piperidine compounds in this group, 19 (70.4%) produced a ratio of ⩾10, and of these, 6 (22.2%) yielded a ratio of ⩾30; among the 20 piperazine compounds in this group, 15 (75%) produced an activity ratio of ⩾10, and of these, 4 (20%) yielded a ratio of ⩾30. It therefore seems that carbonyl-containing piperidines and piperazines exert similar MDR-reversal activity, which again is far superior to that obtained using the corresponding carbonyl-containing, non-cyclic tertiary amine compounds (10 of 11 such compounds produced an MDR-reversal ratio of <10).

In all, 39 of the compounds tested possessed a cyclic tertiary amine and a carbonyl moiety that was located on the bridge between the rings and the amine group or beyond the latter. In 18 of these compounds the carbonyl was part of either an ester bond or an amide of primary or secondary amines. Only 8 of these drugs produced an activity ratio of ⩾10 and none yielded a ratio of ⩾30. In 15 compounds the carbonyl was part of an amide of tertiary amines; among these drugs, 12 produced an MDR-reversal ratio of ⩾10, and of these, 10 yielded a ratio of ⩾30. The MDR-reversal enhancement that results from the addition of an amide of a tertiary amine is further demonstrated by the difference in activity observed between compounds 107 and 209. In 6 compounds the carbonyl was not part of an ester or amide bonds; all 6 of these drugs produced an MDR-reversal ratio of ⩾10 and one of them, compound 144, yielded a ratio of >133.3, which was the highest activity encountered in this study.

These results suggest that an independent carbonyl group or a carbonyl that is part of an amide bond with a tertiary amine (but not with primary or secondary amines) supports MDR-reversal activity, and it seems that such support is not dependent on the exact molecular location of these groups. As compounds possessing such a carbonyl group in the absence of a secondary or tertiary amine function (compounds 44, 87, 203, 221 or 224) were devoid of MDR-reversal activity, it must be concluded that in contrast to the role of the amine group, the carbonyl moiety plays a supportive, albeit non-independent, role. One possible function of the carbonyl group could be mediated by the formation of intra- or intermolecular hydrogen bonds.

The likelihood that a given compound will exert MDR reversal activity in vivo depends not only on its demonstrated in vitro activity but also on its in vivo toxicity, which determines the maximal safe concentration of the compound in the body fluids. Unfortunately, for many of the most active MDR-reversing compounds tested in the present study, such data are not available. However, it seems that the selection of compounds for in vivo studies of MDR reversal should be influenced not only by the magnitude of the reversal obtained in vitro using these compounds but also by the concentration required to achieve the MDR reversal. The relative MDR-reversal index shown in column D of Table 1 takes into account both of these requirements. As shown in column D, compounds 144, 231, 43, 232, 66 and 85 (in decreasing order of activity) yielded the highest relative MDR-reversal indices.

The MDR P388 cells used in the present study have been reported to contain increased levels of P-glycoprotein, a membrane component that is generally believed to be a drug-efflux pump capable of ousting from these cells a rather large variety of drugs [9, 13]. However, we have previously suggested that in these cells, drug resistance may result from reduced drug entry [18]; a similar finding has also been reported in a P-glycoprotein-containing MDR Chinese hamster cell line [21]. It has been suggested that MDR-reversing compounds bind P-glycoprotein in such a fashion that increased drug efflux is inhibited [13]. However, Cass et al. [1] have shown that verapamil treatment sensitises vincristine-MDR cells that lack P-glycoprotein to the same extent as it does cells that contain this glycoprotein. These findings suggest that verapamil can reverse MDR in a manner that is unrelated to its ability to bind P-glycoprotein. We have recently reported that verapamil and two other compounds that reverse MDR induce changes in the lipid composition of MDR but not drug-sensitive P388 cells [19]. Such changes in the membrane lipid composition of MDR cells may lead to an increased rate of drug uptake. Many, if not all, of the active MDR-reversing compounds tested in the present study could be viewed as cationic amphiphilic drugs. As such compounds are known to interfere with cellular lipid metabolism [12], we propose that these activities might be related to their ability to restore drug sensitivity in MDR cells.

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