Structure−Activity Relationship of Newly Synthesized Quinoline Derivatives for Reversal of Multidrug Resistance in Cancer

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The effect of 24 newly synthesized quinoline derivatives on tumor cell multidrug resistance (MDR) was examined *in vitro.* At low concentrations, these compounds enhanced the accumulation of [^{3}H]vincristine in K562/ADM cells and reversed tumor cell MDR. The results of the structure−activity relationship analysis indicate that in highly active compounds the two aryl rings in the hydrophobic moiety deviate from a common plane, so they are capable of interacting with hydrogen bond donors of P-170 glycoprotein (P-gp) *via* π−hydrogen−π interactions. Other major structural features which influence the MDR-reversing activities of these compounds are a quinoline nitrogen atom and a basic nitrogen atom in piperazine. Furthermore, in highly active compounds, the distance between the hydrophobic moiety and the basic nitrogen atom (an atom connected to 2-hydroxypropoxyquinoline) must be at least 5 Å. Several compounds were found to reverse vincristine resistance in K562/ADM cells *in vitro*, and compound **16** (MS-209) was selected for clinical studies.

Introduction

Multidrug resistance (MDR) in cancer chemotherapy is a serious clinical problem. It is well known that P-170 glycoprotein (P-gp), which is expressed on the plasma membrane of drug-resistant tumor cells, actively effluxes antitumor agents out of the cells. The resultant poor accumulation of these agents is the major cause of MDR.[1−5] Verapamil and other calcium channel blockers (dihydropyridine derivatives) are known to reverse MDR.[6−9] Also, a wide variety of other compounds (immunosuppressants, calmodurin antagonists, antihypertensive agents, steroids, and antiparasitic agents) have been shown to reverse MDR *in vitro.*[10] However, the activity of these compounds is low, and various side effects have been observed during clinical trials. Thus, it is necessary to develop more active and less toxic drugs which are capable of reversing MDR of tumor cells.

We studied a number of quinoline derivatives *in vitro* and found that some compounds reverse MDR of tumor cells more effectively than verapamil and exhibit very low toxicity. In particular, compounds **4** (MS-073)[11] and **16** (MS-209)[12] exhibit good pharmaceutical properties. After further examination, MS-209 was selected for clinical studies.

In this report we describe the chemical and pharmacological properties and the structure−activity relationship of newly synthesized quinoline derivatives including MS-209.

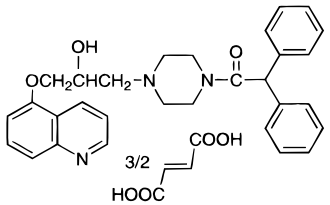

Figure 1. MS-209.

Chemistry

All compounds were prepared according to Scheme 1. The preparation of amine moieties is depicted in Scheme 2. The alkylpiperazine derivatives listed in Tables 1 and 2 were prepared by method A, acylpiperazine derivatives by method B, and piperidine derivatives by method C. The preparation of typical compounds is described in the Experimental Section.

Computational Analysis

The structures of the compounds studied were either assigned using the experimental data for similar compounds stored in the Cambridge Structure Database (the CSD)[13] or calculated using the molecular mechanics MM2[14] method. "Du" denotes a centroid of two aromatic rings in the hydrophobic moiety (Figure 3).

Pharmacology

The effect of quinoline derivatives was tested *in vitro* on K562/ADM cells at 1 μM concentration of [^{3}H]-VCR (vincristine). The intracellular [^{3}H]VCR was assayed by measuring the cell radioactivity. The drug activities relative to verapamil were calculated using

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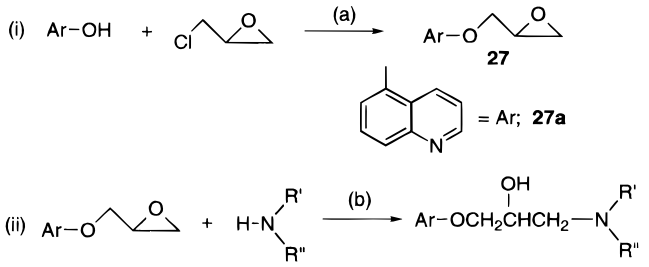
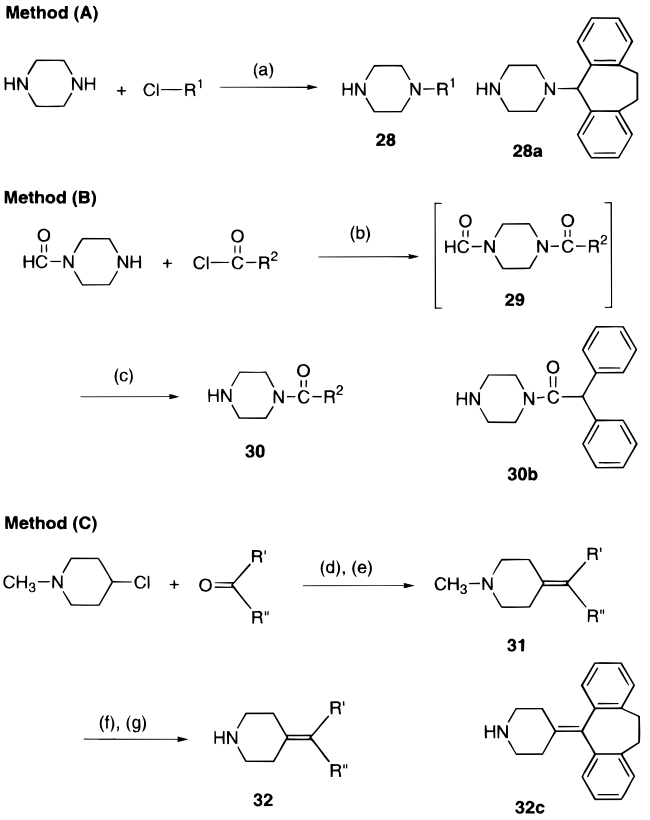
Scheme 1[a]



[a] Reagents: (a) NaH in DMF; (b) reflux in EtOH.

Scheme 2[a]



[a] Reagents: (a) reflux in dioxane; (b) Et_3N in $CHCl_3$; (c) HCl in MeOH; (d) Mg in THF/Tol; (e) CF_3COOH; (f) CCl_3CH_2OCOCl/K_2CO_3 in Cl_2CHCH_2Cl; (g) Zn/NH_4Cl in THF.

the following formula:

$$[([^3H]\text{VCR accumulation value of drug}) + ([^3H]\text{VCR accumulation value of verapamil})] \times 100 = \text{activity (\%)}$$

The compounds were classified as follows: high activity, >301%; good activity, 300−201%; moderate activity, 200−101%; weak activity, <100%. Effects of MS-209 and verapamil are described in the Experimental Section.

Results and Discussion

We found that quinoline derivative **1** strongly enhanced the accumulation of [^{3}H]VCR in K562/ADM cells (Table 1). The MDR-reversing activity of compound **1** was 3−4 times higher than that of verapamil *in vitro*. To develop highly active MDR-reversing drugs with low toxicity, we analyzed the structure−activity relationship of quinoline derivatives.

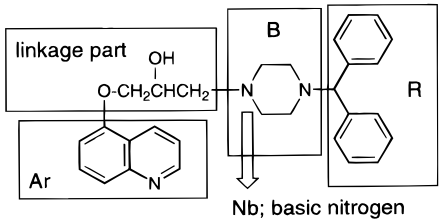
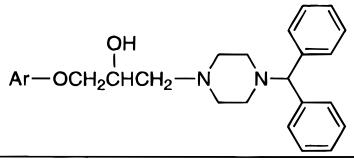


Figure 2. Structural features of quinoline derivatives.

Table 1. Aryl Ring Derivatives



Compd	Ar -	Activity[a]	Formula
1	5-quinolyl	358	$C_{29}H_{31}N_3O_2$
2	1-naphthyl	32	$C_{30}H_{32}N_2O_2$
3	C_6H_5-	56	$C_{26}H_{30}N_2O_2 \cdot HCl$

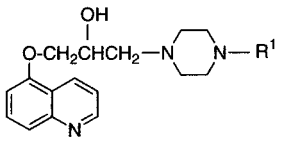
[a] Activity: relative to verapamil (%).

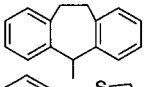
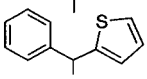
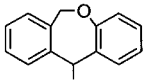
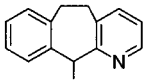
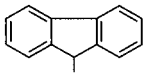
The following structural features of compound **1** may be related to its MDR-reversing activity: (i) a quinoine ring, (ii) a linkage moiety (2-propanol moiety), (iii) a piperazine ring, and (iv) a hydrophobic moiety (Figure 2). When the quinoline ring in compound **1** was replaced by a naphthyl ring or a phenyl ring, the resulting derivatives, **2** and **3**, were found to be significantly less active than verapamil. Thus, the quinoline moiety seemed to play an important role in determining the drug activity.

To study the effect of the hydrophobic moiety, we synthesized 20 piperazine and piperidine derivatives. The structure−activity data of *N*-alkyl- and *N*-acylpiperazine derivatives are presented in Tables 2 and 3, and the data of alkenylpiperidine derivatives are in Table 4. The MDR-reversing activities of an unsubstituted compound (**15**, Table 2) and *N*-aliphatic derivatives **13** and **14** were weak (<100%). Also the activity of monoaromatic derivative **12** was moderate (123%) compared to diaryl derivatives **1**, **4**−**11**, and **16**−**22** (Tables 2−4). Therefore, high activities of the studied compounds seemed to be related to the presence of a pair of aryl rings. Among the derivatives with such a hydrophobic moiety, diphenylmethyl- (**1** and **16**), dibenzocycloheptano, (**4** and **20**), α-thienylbenzyl- (**5**), dibenzoxepino- (**6**), and benzocycloheptanopyridine (**7**) showed the highest activity (>301%). Chlorophenyl (**8**), bis(fluorophenyl) (**9**, **18**, and **22**), bis(methoxyphenyl) (**10**), fluorenyl (**11** and **19**), and xanthenyl (**17**) derivatives were less active (300−201%).

Zamora *et al.*[15] who studied the structure−activity relationship of some MDR-reversing drugs have already emphasized the importance of aromatic rings in hydrophobic parts of the drugs. According to our results (Table 2−4), the activity of the compounds examined depends not only on the presence of aryl rings in hydrophobic moieties but also on relative ring positions. For example compounds **1**, **16**, and **21**, which contained two phenyl rings, each of which deviated by about 80° from the plane, were more active than planar fluorene (**11** and **19**) or xanthene (**17**) derivatives (Figure 3). Also,

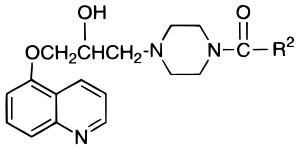
Table 2. *N*-Alkylpiperazine Derivatives

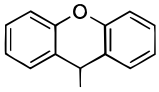
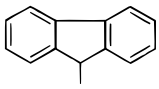


Compd	-R^1	Activity[a]	Formula
1	-$CH(C_6H_5)_2$	358	$C_{29}H_{31}N_3O_2$
4		371	$C_{31}H_{33}N_3O_2$
5		428	$C_{27}H_{29}N_3O_2S$
6		414	$C_{30}H_{31}N_3O_3{\cdot}3HCl{\cdot}2H_2O$
7		316	$C_{30}H_{32}N_4O_2{\cdot}1.2H_2O$
8	-$CH(C_6H_5)(4\text{-}Cl\text{-}C_6H_4)$	243	$C_{29}H_{30}N_3O_2Cl{\cdot}3HCl{\cdot}2.5H_2O$
9	-$CH(4\text{-}F\text{-}C_6H_4)_2$	265	$C_{29}H_{29}N_3O_2F_2{\cdot}3HCl{\cdot}2.5H_2O$
10	-$CH(4\text{-}CH_3O\text{-}C_6H_4)_2$	260	$C_{31}H_{35}N_3O_4{\cdot}2.5HCl{\cdot}3H_2O$
11		229	$C_{29}H_{29}N_3O_2{\cdot}H_2O$
12	-$CH_2C_6H_5$	123	$C_{23}H_{27}N_3O_2$
13	-$(CH_2)_{17}CH_3$	55	$C_{34}H_{57}N_3O_2{\cdot}0.5H_2O$
14	-CH_3	47	$C_{17}H_{23}N_3O_2{\cdot}0.5H_2O$
15	-H	65	$C_{16}H_{21}N_3O_2{\cdot}3HCl{\cdot}1.5H_2O$

[a] See corresponding footnote in Table 1.

Table 3. *N*-Acylpiperazine Derivatives

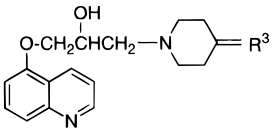


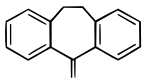
Compd	- R^2	Activity[a]	Formula
16	-$CH(C_6H_5)_2$	381	$C_{30}H_{31}N_3{\cdot}1.5C_4H_4O_4$
17		279	$C_{30}H_{29}N_3O_4{\cdot}0.5H_2O$
18	-$CH(4\text{-}F\text{-}C_6H_4)_2$	263	$C_{30}H_{29}N_3O_3F_2{\cdot}0.2H_2O$
19		213	$C_{30}H_{29}N_3O_3{\cdot}1H_2O$

[a] See corresponding footnote in Table 1.

other highly active compounds such as dibenzocycloheptano- (**4** and **20**, 56−57°), dibenzoxepino- (**6**, 43°), and benzocycloheptanopyridine (**7**, same as **4**) were nonplanar (Figure 3). Thus it seems that the deviation of the two phenyl rings from planarity is an essential feature of highly active compounds. If hydrogen bond donors of P-gp are set between two deviated aromatic rings in the hydrophobic moieties (π-hydrogen-π interactions), then the enhanced activity of compounds having nonplanar aryl rings can be due to the π-hydrogen-π interactions. Such interactions do not seem to be rare. We find the same type of interactions in

Table 4. Substituted Piperidine Derivatives



Compd	= R^3	Activity[a]	Formula
20		374	$C_{32}H_{32}N_2O_2$
21	$=C(C_6H_5)_2$	309	$C_{30}H_{30}N_2O_2{\cdot}H_2O$
22	$=CH(4\text{-}F\text{-}C_6H_4)_2$	246	$C_{30}H_{28}N_2O_2F_2{\cdot}0.2H_2O$

[a] See corresponding footnote in Table 1.

molecules: JOSVUI,[16] JIDPUH,[17] and others (CSD).[13] In these compounds, the diphenylmethyl groups interact with the intermolecular primary amine (JOSVUI) or water (JIDPUH), and the distances between each centroid of the phenyl ring and the amine nitrogen or the oxygen of water hydrogen bonding with phenol are about 3.8 Å (with nitrogen) and 3.2 Å (with oxygen). Such interactions have been observed in a substance P antagonist (CP96345),[18] in which the diphenylmethyl groups binds specifically to histidine 197 of the human neurokinin-1 receptor.

Although steric bulkiness and hydrophobicity in unsubstituted nonplanar compounds (**1**, **16**, and **21**, have activity, >301%) and substituted nonplanar compounds (**8**−**10**, **18**, and **22**, good activity, 300−201%) are not very different, the activities differ significantly. This is consistent with the π−hydrogen−π interaction hypothesis described above. For example, according to the *ab initio* molecular orbital study,[19] a complex formed by an ammonium cation and a fluorobenzene molecule is less stable than a complex formed by an ammonium cation and a benzene molecule. Consequently, the π−hydrogen−π interactions in **8**−**10**, **18**, and **22** are expected to be weaker than in **1**, **16**, and **21**, which agrees with the observed MDR-reversing activity trends (Tables 2−4).

The MDR-reversing activity is also affected by the distance between the hydrophobic moiety and the basic nitrogen, Nb, of piperazine (Figure 2). In the most active compounds **1**, **23**, and **24** (high activity, >301%) these distances are larger than 5 Å, whereas in **25** (moderate activity, 117%) it is about 4 Å (Figure 4). On the other hand, a comparison between the activity of compound **4** (high activity, 371%) which has the basic nitrogen, Nb, in its piperazine moiety and that of compound **26** (weak activity, 81%), whose piperazinedione nitrogen atoms are much less basic than the piperazine Nb nitrogen, indicates that the basicity of the nitrogen atom within the piperazine moiety is required for MDR-reversing activity.

We have previously reported that compound **4** was about 20 times more active than verapamil in IC_{50} value and completely reversed MDR at 3 μM concentration on K562/ADM cells.[11] Although the *in vitro* activity of **16** was lower than that of compound **4**, its *in vivo* property is better than that of compound **4**.[12] Therefore, compound **16** has been selected for clinical studies due to its promising properties, high activity, and low toxicity in *in vivo* studies.[12]

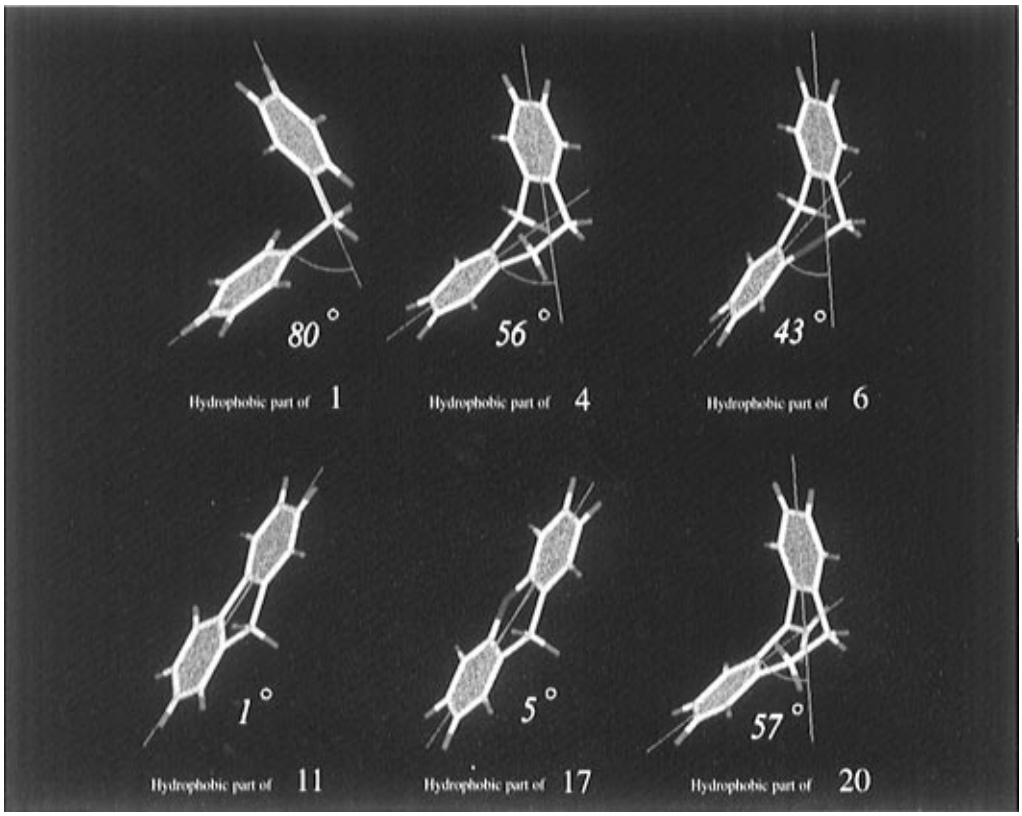


Figure 3. Deviation from planarity of two aromatic rings in the hydrophobic moiety.

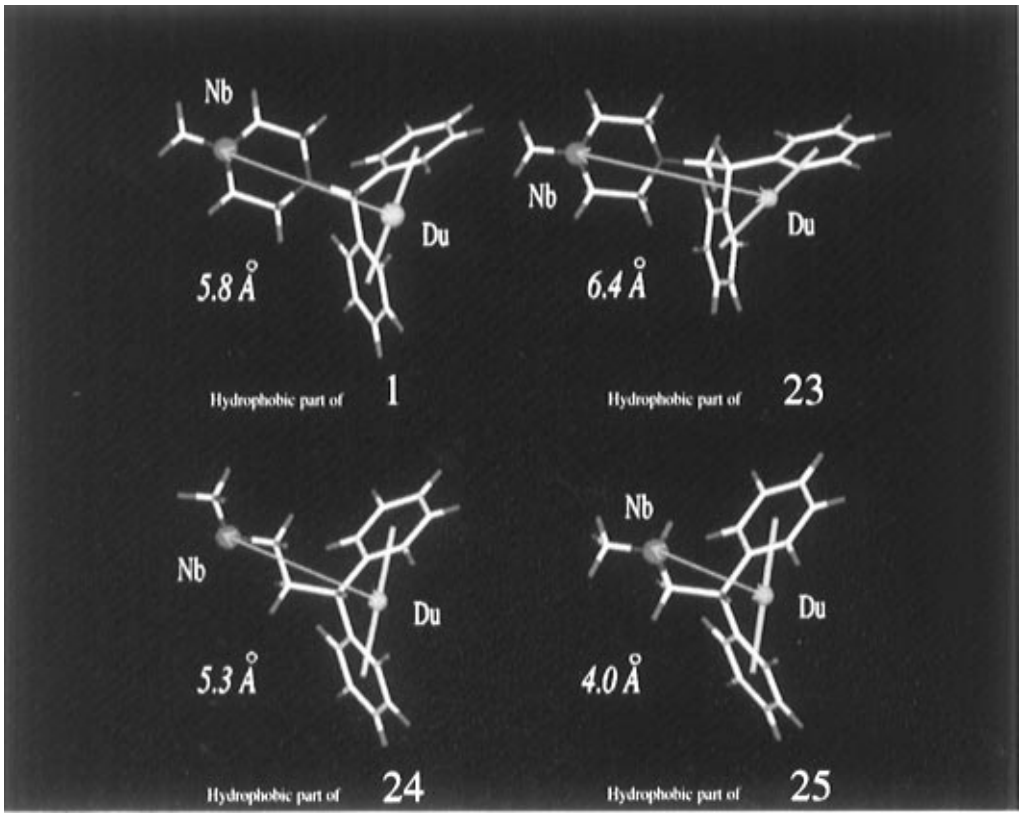
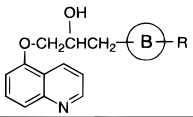


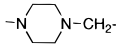
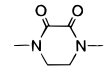
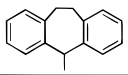
Figure 4. Distance between the basic nitrogen (Nb) and the hydrophobic moiety (Du).

Conclusions

Studies regarding the structure−activity relationship of a number of quinoline derivatives allowed us to develop highly active MDR-reversing drugs. The results obtained indicate that the deviation of two aryl rings in the hydrophobic moiety is essential for the effective reversal of tumor cell MDR. The analysis of other structural factors which affect the interactions between the drug and P-gp confirmed that in highly active compounds basic nitrogen atoms, in the piperazine moiety (Nb in Figure 2) and in the quinoline moiety, are indispensable. Another prerequisite of high activity

Table 5. Distance and Basicity of Nitrogen (Nb)



Compd	B	R	Activity[a]	Formula
23		$-CH(C_6H_5)_2$	327	$C_{30}H_{33}N_3O_2 \cdot 1HCl$
24	$-NHCH_2CH_2-$	$-CH(C_6H_5)_2$	343	$C_{26}H_{26}N_2O_2 \cdot 1.9HCl \cdot 1.5H_2O$
25	$-NHCH_2-$	$-CH(C_6H_5)_2$	117	$C_{27}H_{28}N_2O_2 \cdot 1.5HCl \cdot 1.1H_2O$
26			81	$C_{31}H_{29}N_3O_4 \cdot 2HCl \cdot 1.5H_2O$

[a] See corresponding footnote in Table 1.

is the requirement that the distance between the basic nitrogen atoms and hydrophobic moieties is at least about 5 Å.

Experimental Section

Chemistry. Melting points were obtained on a Buchi capillary melting point apparatus. ^{1}H-NMR spectra were obtained using a JEOL EX-270 instrument, and infrared spectra were recorded with a JASCO IRA-2 spectrometer using KBr pellets. Elemental analyses were within 0.4% of the theoretical values, and structural assignments were consistent with NMR and IR spectra.

Method A To Obtain *N*-Alkyl Derivatives: 5-[3-{4-(10,-11-Dihydro-5*H*-dibenzo[*a*,*d*]cycloheptan-5-yl)piperazin-1-yl}-2-hydroxypropoxy]quinoline (4, MS-073). (i) *N*-(10,-11-Dihydro-5*H*-dibenzo[*a*,*d*]cycloheptan-5-yl)-piperazine (28a). 5-Chlorodibenzosuberane (5.0 g, 21.9 mmol) was added to anhydrous piperazine (11.3 g, 131 mmol) in 80 mL of dioxane and heated under reflux for 7 h. After it cooled to room temperature, insoluble salts were removed by filtration, and the solvent was distilled off. A small amount of petroleum ether was added for crystallization, and crystalline **28a** was collected (5.1 g, 84%): mp 110−111.5 °C; ^{1}H-NMR ($CDCl_3$) δ 2.1−2.4 (brs, 4H), 2.7−2.9 (m, 6H), 3.9−4.1 (m, 2H), 3.95 (s, 1H), 3.9−4.1 (m, 2H), 7.0−7.3 (m, 8H); IR (KBr) 3420, 3250, 2920, 2800, 1630, 1490, 1450, 1330, 1140 cm^{-1}.

(ii) 5-(2,3-Epoxypropoxy)quinoline (27a). 5-Hydroxyquinoline (1 g, 6.9 mmol) was added to a solution of potassium *tert*-butoxide (0.93 g, 8.3 mmol) in dry DMF (20 mL), and the resulting solution was heated at 50 °C for 30 min. After the solution was allowed to cool to room temperature, epichlorohydrin (1.92 g, 20.75 mmol) was added, and the mixture was heated at 90 °C for 3 h. The solvent was distilled off under reduced pressure at a temperature below 50 °C. Ice−water was added to the residue and then extracted with ethyl acetate three times. Then the organic layer was dried and evaporated, to give **27a** as a reddish oil (0.88 g, 53%, unstable): ^{1}H-NMR ($CDCl_3$) δ 2.85 (dd, 1H, J = 2.9, 5.2 Hz), 2.97 (dd, 1H, J = 5.2, 9.5 Hz), 3.45−3.5 (m, 1H), 4.11 (dd, 1H, J = 5.9, 11 Hz), 4.45 (dd, 1H, J = 2.9, 11 Hz), 6.87 (d, 1H, J = 8.1 Hz), 7.72 (d, 1H, J = 8.1 Hz), 8.64 (d, 1H, J = 8 Hz), 8.91 (dd, 1H, J = 1.5, 4.4 Hz).

(iii) 5-[3-{4-(10,11-Dihydro-5*H*-dibenzo[*a*,*d*]cycloheptan-5-yl)piperazin-1-yl}-2-hydroxypropoxy]quinoline (4, MS-073). Compounds **27a** (0.88 g, 4.4 mmol) and **28a** (1.2 g, 4.3 mmol) were dissolved in 20 mL of ethanol and were under reflux for 3 h. Then the solvent was distilled off, and the residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 50/1) and recrystallized from EtOH, to give compound **4** (1.4 g, 66%): mp 126−128 °C; ^{1}H-NMR ($CDCl_3$) δ 2.2−2.9 (m, 12H), 3.1−3.6 (brs, 1H), 3.9−4.3 (m, 6H), 6.86 (d, 1H, J = 8.0 Hz), 7.0−7.2 (m, 8H), 7.36 (dd, 1H, J = 4.4, 8.1 Hz), 7.59 (t, 1H, J = 8.0 Hz), 7.69 (d, 1H, J = 8.1 Hz), 8.57 (d, 1H, J = 8.0 Hz), 8.90 (dd, 1H, J = 1.5, 4.4 Hz); IR (KBr) 2900, 2800, 1620, 1590, 1570, 1450, 1260, 1140, 1100 cm^{-1}.

Method B To Obtain *N*-Acyl Derivatives: 5-[3-{4-(2,2-Diphenylacetyl)piperazin-1-yl}-2-hydroxypropoxy]quinoline 1.5Fumarate (16, MS-209). (i) *N*-(2,2-Diphenylacetyl)piperazine (30b). 2,2-Diphenylacetyl chloride (125 g, 0.542 mol) in dry $CHCl_3$ (350 mL) was slowly added to a solution of *N*-formylpiperazine (64.8 g, 0.568 mol) and triethylamine (66 g, 0.65 mol) in dry $CHCl_3$ (350 mL) immersed in an ice bath. The reaction mixture was stirred for 30 min at room temperature. Then the resultant solution of **29b** was washed with 0.1 N HCl solution (500 mL), 0.1 N NaOH solution (500 mL), and saturated NaCl solution (500 mL). To the $CHCl_3$ solution of **29b** was added 10% HCl/methanol solution (1000 mL), and the mixture was allowed to stand at room temperature overnight. About 775 mL of NaOH solution was added to adjust the pH of the final solution to the range of 8−9. The organic layer was separated and washed with saturated NaCl aqueous solution, then dried, and evaporated to give **30b** (136.6 g, 90%): mp 83−84 °C; ^{1}H-NMR ($CDCl_3$) δ 2.5−2.6 (m, 2H), 2.8−2.9 (m, 2H), 3.4−3.5 (m, 2H), 5.19 (s, 1H), 7.2−7.4 (m, 10H); IR (KBr) 2833, 1634, 1434, 1218, 1040, 782, 746, 700 cm^{-1}.

(ii) 5-[3-{4-(2,2-Diphenylacetyl)piperazin-1-yl}-2-hydroxypropoxy]quinoline. Compounds **27a** (4.4 g, 21.9 mmol) and **30b** (6.50 g, 23.2 mmol) were dissolved in 85 mL of IPA and heated under reflux for 4 h. Then the solvent was distilled off, and the residue was purified by short silica gel column chromatography to give the free form of **16** (7.37 g, 70%): mp 161−162 °C; ^{1}H-NMR ($CDCl_3$) δ 2.2−2.8 (m, 6 H), 3.5−3.6 (m, 2H), 3.7−3.9 (m, 2H), 4.1−4.3 (m, 3H), 5.20 (s, 1H), 6.86 (d, 1H, J = 7.3 Hz), 7.2−7.4 (m, 11H), 7.59 (t, 1H, J = 8.1 Hz), 7.71 (d, 1H, J = 8.1 Hz), 8.54 (d, 1H, J = 7.3 Hz), 8.91 (dd, 1H, J = 2, 4 Hz); IR (KBr) 2954, 1630, 1587, 1268, 1091, 802, 748, 703 cm^{-1}.

(iii) 5-[3-{4-(2,2-Diphenylacetyl)piperazin-1-yl}-2-hydroxypropoxy]quinoline 1.5Fumarate (16, MS-209). The free form of **16** (1.22 g, 2.54 mmol) was added to a solution of fumaric acid (0.89 g, 7.67 mmol) in MeOH (15 mL) and allowed to stand at room temperature for 6 h. Crystals were collected by filtration and washed with MeOH. Then the crystals were recrystallized from MeOH (26 mL) to give **16** (1.0 g, 60%): mp 210 °C dec; ^{1}H-NMR (DMSO-d_6) δ 2.2−2.6 (m, 6H), 3.4−3.6 (m, 4H), 4.0−4.2 (m, 3H), 5.53 (s, 1H), 6.63 (s, 3H), 7.03 (d, 1H, J = 8.1 Hz), 7.2−7.4 (m, 10H), 7.5−7.7 (m, 3H), 8.61 (d, 1H, J = 8.1 Hz), 8.89 (dd, 1H, J = 1.5, 4.4 Hz); IR (KBr) 3424, 1644, 1592, 1277, 1180, 1110, 799 cm^{-1}.

Method C To Obtain Piperidine Derivatives: 5-[3-{4-(10,11-Dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-yl)piperidin-1-yl}-2-hydroxypropoxy]quinoline (20). (i) 4-(10,11-Dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-yl)-*N*-methylpiperidine (31c). 4-Chloro-1-methylpiperidine (7.5 g, 56 mmol) in dry THF (10 mL) was added to a suspension of Mg (1.34 g) in dry THF (18 mL), and the mixture was refluxed for 1 h. The reaction mixture was cooled to 0 °C, dibenzosuberone (9.4 g, 45 mmol) in toluene (10 mL) was added, and the solution was warmed to 40 °C. The reaction mixture was poured into dilute HCl solution and washed with Et_2O (150 mL). Then the aqueous layer was neutralized with saturated $NaHCO_3$ solution and extracted with CH_2Cl_2 (three times, 500 mL). The organic layer was dried and concentrated, and the resulting residue was crystallized by the addition of Et_2O. The crystals were collected by filtration, washed with Et_2O, and then dissolved in CF_3COOH (15 mL) and allowed to stand overnight at room temperature. The solution was added to cold dilute NaOH solution, and the resulting basic solution was extracted with CH_2Cl_2 three times. Then the solution was dried and evaporated to give **31c** (4.9 g, 36%): ^{1}H-NMR ($CDCl_3$) δ 2.0−2.2 (m, 2H), 2.3 (s, 3H), 2.4−2.5 (m, 4H), 2.6−2.7 (m, 2H), 2.75−2.9 (m, 2H), 7.0−7.1 (m, 8H).

(ii) 4-(10,11-Dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-yl)piperidine (32c). A solution of **31c** (1.45 g, 5 mmol) and 2,2,2-trichloroethyl chloroformate[20] (2.1 g, 10 mmol) was stirred under reflux with potassium carbonate (1.03 g, 7.5 mmol) in 1,1,2-trichloroethane (80 mL) for 11 h; then MeOH (1 mL) was added and the solution concentrated. The residue was dissolved in a solution of 1 M NH_4Cl (4 mL) and THF (20 mL), and then zinc powder (4 g) was added. The solution was

stirred at room temperature for 24 h, and a mixture of CH_2Cl_2 and MeOH was added. Then insoluble substances were filtered off, and the filtrate was dried and evaporated to give **32c** (660 mg, 48%): mp >240 °C; ^{1}H-NMR ($CDCl_3$) δ 2.6−3.0 (m, 8H), 3.3−3.4 (m, 4H), 7.0−7.2 (m, 8H), 9.8 (brs, 1H); IR (KBr) 2901, 1556, 1441, 1303, 1032, 1001, 777, 754 cm^{-1}.

(iii) 5-[3-{4-(10,11-Dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-yl)piperidin-1-yl}-2-hydroxypropoxy]quinoline (20). Et_3N (2 mL) was added to a solution of **32c** (650 mg, 2.4 mmol) and **27a** (520 mg, 2.64 mmol) in EtOH (100 mL) and heated under reflux for 9 h. Then the solution was concentrated and purified by silica gel column chromatography ($CHCl_3$/MeOH = 30/1) to give **20** (400 mg, 36%): ^{1}H-NMR ($CDCl_3$) δ 2.3−3.0 (m, 12H), 3.3−3.5 (m, 2H), 4.1−4.3 (m, 3H), 6.88 (d, 1H, J = 7.4 Hz), 7.0−7.2 (m, 8H), 7.37 (dd, 1H, J = 4.0, 8.4 Hz), 7.61 (t, 1H, J = 7.4 Hz), 7.69 (d, 1H, J = 8.4 Hz), 8.58 (d, 1H, J = 7.4 Hz), 8.85 (dd, 1H, J = 1.5, 4.0 Hz); IR (KBr) 3400, 2920, 1630, 1590, 1410, 1280, 1100, 790 cm^{-1}.

Pharmacology. Cellular Accumulation of [^{3}H]VCR. Cell suspensions of K562/ADM (1.5×10^6/mL) in the growth medium with 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer were incubated at 37 °C with 30 nM [^{3}H]-VCR (5.6 Ci/mmol) in the presence (1 μM) or absence of MS-209 and verapamil. At various intervals, the amount of intracellular [^{3}H]VCR was determined as described previously.[21] In brief, after the resuspension, 0.5-mL aliquots were transferred onto an oil layer consisting of Toray Silicon SH550 (Toray Silicon Co., Ltd., Tokyo, Japan) and liquid paraffin (Wako Pure Chemical Industries, Ltd., Tokyo, Japan) at a volume ratio of 4:1 in a 1.5-mL microtube. After centrifugation, the supernatant fluid was removed. The cell pellet was then lyzed overnight with 0.25 mL of 0.5 N KOH solution, and the radioactivity was counted in a Beckman LS1701 liquid scintillation system after the addition of 10 mL of Aquasol (New England Nuclear, Boston, MA). Radioactivities of control, verapamil, and MS-209 were 958, 2137, and 8164 dpm. Activity of MS-209 relative to verapamil was calculated as 381%.

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