New Purines and Purine Analogs as Modulators of Multidrug Resistance

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A series of 36 purine and purine analog derivatives have been synthesized and tested for their ability to modulate multidrug resistance in vitro (P388/VCR-20 and KB-A1 cells) and in vivo (P388/VCR leukemia). Compounds were compared to S9788, a triazine derivative which has already shown some activity during phase 1 clinical trials and also a limiting cardiovascular side effect possibly linked to its calcium channel affinity. The fact that active compounds increase adriamycin accumulation in the resistant KB-A1 cells, and not in the sensitive KB-3-1 cells, suggests they act predominantly by inhibiting the P-glycoprotein-catalyzed efflux of cytotoxic agents. No direct relation was found between the affinity for the phenylalkylamine binding site of the calcium channel and in vitro sensitization of resistant cells. In vivo, when administered po in association with vincristine (0.25 mg/kg), five compounds (**3**, **4**, **9**, **25**, and **26**), of very differing calcium channel affinities (K_i from 5 to 560 nM), fully restored (T/V ≥ 1.4) the sensitivity of P388/VCR leukemia to vincristine.

The resistance of tumor cells to cytotoxic drugs is a major problem in cancer chemotherapy. The implicated mechanisms are intrinsic or acquired. Among them, multidrug resistance (MDR), associated with the membrane P-glycoprotein overexpression, has been defined as a cross-resistance to anticancer drugs especially affecting vinca-alcaloides, anthracyclines, podophyllotoxins, actinomycin D, taxol, and colchicine.

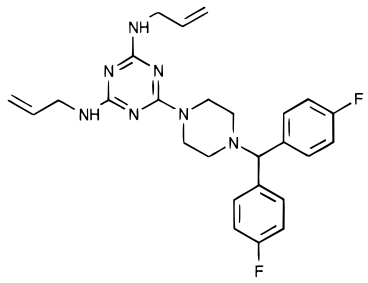
By systematic screening, we found that almitrine (**1**), a triazinylpiperazine currently used for respiratory insufficiency, moderately sensitized the highly resistant cell line DC-3F/AD to actinomycin D. This starting point led the synthesis of a series of analogs in order to select more potent modulator agents[1] and provided S9788 (**2**), which is currently in Phase 1 clinical trials.

The preliminary clinical trials demonstrated the interest of S9788 in modulating resistance. However, the observed limiting toxicity is that of cardiovascular side effects including prolonged QTc intervals.[2,3] Consequently, active plasma levels could not be achieved in the patients. Moreover, S9788 was shown to have calcium channel affinity comparable to that of verapamil and markedly higher than that of almitrine. While the clinical syndrome of long QT and Torsades de Pointes is not generally associated with calcium channel blockers therapy,[4,5] marked proarythmic effects were observed with some calcium antagonists, such as bepridil, that prolonged ventricular repolarization and QT intervals.[6,7]

These results prompted us to search for second-generation sensitizers, more efficient and more potent than S9788 in vitro and in particular more active in vivo by oral administration. These modulator agents also would have to present a lower affinity for the phenylalkylamine binding site of the calcium channel, to attempt to reduce cardiovascular side effects observed with S9788.

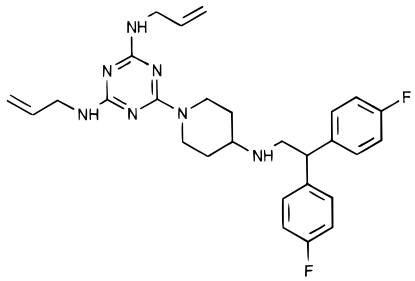
This paper describes the chemical properties of new compounds, their ability to modulate MDR in vitro and in vivo, and their calcium channel affinity. These new compounds have the general formula shown in Chart 3. The formula partially respects structure−activity relationships established from the first series of compounds, including S9788.[1] In the central part B, Z can be CHNH (4-aminopiperidine) or N (piperazine), $m = 0$ or 1. In part C, the two phenyl rings can eventually be linked in a bridge including carbons and/or one or several heteroatoms. T and V, alternatively or together, represent H or halogen atoms or methoxy radicals. The major modification concerns part A in which the S9788 triazine core is replaced by a purine core or an analog in which the triaminotriazine arrangement remains unchanged. The substituents on positions 2 and 6 can be exchanged. R and R′ represent alternatively or

Chart 1



almitrine **1**

Chart 2



S 9788 **2**

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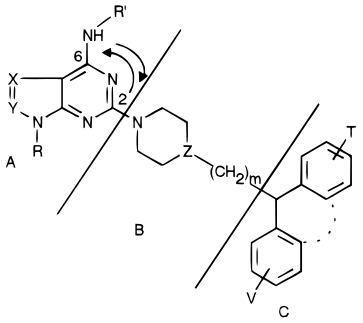
Chart 3



together an alkyl or alkenyl group and X and Y a carbon or nitrogen atom.

Chemistry

The target compounds listed in Tables 1−3 have been prepared according to the Schemes 1−3. Table 4 and Schemes 4 and 5 are related to the starting materials.

Alkylation of 2,6-dichloropurine by means of Mitsunobu's method[8] afforded a mixture of 7- and 9-isomers which were separated and purified by flash chromatography (Scheme 4). To avoid reproduction of this isomer mixture in the case of triazolopyrimidines, the alkylation was performed on the precursor pyrimidine before cyclization (Scheme 5). Dichloropyrrolopyrimidine[9] and dichloropyrazolopyrimidine,[10] which have been already described, were alkylated by the same Mitsunobu method (Scheme 4).

Results and Discussion

Structure−Activity Relationships (SAR). For the discussion of SAR, the general formula has been divided into three parts (see Chart 3). To obtain a potent and optimal MDR modulation on P388/VCR-20 and/or KB-A1 cells lines at nontoxic concentrations in this series (see Table 5), the following criteria have emerged.

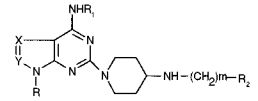
(1) Part A should preferably be a pyrrolopyrimidine core (XY = CC, **18**) rather than a purine (XY = NC, **3**), pyrazolopyrimidine (XY = CN, **13**), or triazolopyrimidine core (XY = NN, **27**), other components being constant. R and R′ groups (see Chart 3) can be equally allyl and/or propyl ones, the reversing activity on both cells lines being of the same order (compare **3**, **24**, **25**, and **26**). Permutation of substituents on positions 2 and 6 (see Chart 3) do not greatly modify compound activities (compare **4** vs **29**, **6** vs **30**, **7** vs **31**, **8** vs **32**, **9** vs **33**, and **10** vs **34,** respectively).

(2) Part B should be a 4-(methylamino)piperidine group ($m = 1$) rather than a 4-aminopiperidine ($m = 0$) (compare **8** vs **16** and **3** vs **19**), and when Part B is a piperazine, the compounds are almost inactive (see **36**−**38**).

(3) Concerning part C, steric factors do not seem to be determinant as activities of the same order are obtained with a tricyclic (**10**), a bicyclic (**7**, **21**), a benzhydryl (**5**), or a triphenylmethyl group (**20**). A tricyclic group with a heteroatomic bridge affords slightly better compounds than a carbon bridge, at least in the case of KB-A1 cells (compare **8** and **17** vs **3** and **10**).

For the discussion concerning calcium channel affinity, the general formula has again been divided into the same three parts. Compounds **16**, **19**, **29**, **and 35**−**38**, which are less active than S9788, will not be discussed.

Table 1. Target Compounds: (Alkylamino)purines and Analogs

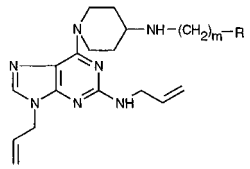


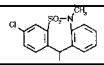
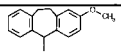
n.	R	R1	R2	m	X	Y	mp,°C	cryst solvent	formula[a]
3[b]				1	N	C	142-4	Et_2O	$C_{32}H_{37}N_7$
4				1	N	C	143-5	C_6H_6	$C_{30}H_{33}F_2N_7$
5				1	N	C	150	EtOH	$C_{30}H_{35}N_7$
6				1	N	C	174	EtOH	$C_{31}H_{35}ClN_8O_2S$, $C_4H_4O_4$[d]
7				1	N	C	222	EtOH	$C_{27}H_{31}N_7$, $C_4H_4O_4$[d]
8				1	N	C	240	EtOH	$C_{31}H_{35}N_7O$, $C_4H_4O_4$[d]
9				1	N	C	205	EtOH	$C_{33}H_{39}N_7O$, $C_4H_4O_4$[d]
10				1	N	C	250	EtOH	$C_{32}H_{35}N_7$, $C_4H_4O_4$[d]
11[f]				0	N	C	175	EtOH, Et_2O	$C_{30}H_{33}ClN_8O_2S$, 2 HCl
12[e,g]				0	N	C	210	EtOH, Et_2O	$C_{30}H_{33}ClN_8O_2S$, 2 HCl
13				1	C	N	230 (dec)	EtOH	$C_{32}H_{37}N_7$, 1.2 $C_4H_4O_4$[d]
14[c]				1	N	C	165	EtOH	$C_{33}H_{40}N_8O_2S$
15[c]				1	N	C	170	EtOH	$C_{35}H_{43}N_7O_3$, 1.5 $C_4H_4O_4$[d]
16				0	N	C	223	EtOH	$C_{30}H_{33}N_7O$, 1.5 $C_4H_4O_4$[d]
17				1	N	C	212-5	EtOH	$C_{31}H_{34}N_8O$
18				1	C	C	160-3	EtOH	$C_{33}H_{38}N_6$, 0.5 $C_4H_4O_4$[d]
19				0	N	C	114	Et_2O	$C_{31}H_{35}N_7$
20				1	N	C	175	EtOH	$C_{36}H_{39}N_7$
21				1	N	C	203	EtOH	$C_{28}H_{37}N_7$, $C_4H_4O_4$[d]
22				1	C	N	215-220	EtOH	$C_{33}H_{41}N_7O$, $C_4H_4O_4$[d]
23				1	C	N	207-212	EtOH	$C_{33}H_{39}N_7O$, 1.2 $C_4H_4O_4$[d]
24				1	N	C	237	EtOH	$C_{32}H_{39}N_7$, $C_4H_4O_4$[d], 0.5 H_2O
25				1	N	C	213	EtOH	$C_{32}H_{39}N_7$, $C_4H_4O_4$[d]
26				1	N	C	106-112	Et_2O	$C_{32}H_{41}N_7$
27				1	N	N	178-0	EtOH/ CH_2Cl_2 9/1	$C_{31}H_{36}N_8$

[a] All compounds except **9**, **10**, **33**, and **34** were purified by flash chromatography. C, H, N, analyses were within 0.4% of theoretical values for the formulae given, except for the following products. **8**: N calcd, 15.37; found, 14.95. **11**: Cl calcd, 15.69; found 15.28. **14**: H calcd, 6.58; found, 7.05. **21**: H calcd, 7.03; found, 6.58. All compounds exhibited NMR consistent with assigned structures. [b] Synthesis described in the Experimental Section. [c] For starting materials, see the Experimental Section. [d] Fumaric acid. [e] Maleic acid. [f] $[\alpha]_D = +34.6°$ ($c = 0.5\%$, EtOH, $t = 21$ °C). [g] $[\alpha]_D = -34.8°$ ($c = 0.5\%$, EtOH, $t = 21$ °C).

(1) Part A: Compound affinities depend on the nature of the heteroaromatic core, other components being constant. The order of decreasing affinities is **3** (XY = NC) > **18** (XY = CC) > **13** (XY = CN) > **27** (XY = NN). When R and R′ (see Chart 3) are the same group, the highest affinity is observed for the allyl group, and for the propyl group, the affinity is actually divided by about 12 (compare **26** vs **3**). Except for **33** vs **9**, permutation of the residues at positions 2 and 6 (see

Table 2. Target Compounds: 6-Piperidinopurines



n.	R	m	mp,°C	cryst solvent	formula[a]
28		1	204	EtOH	$C_{32}H_{37}N_7$, 2.5 $C_4H_4O_4$[d]
29		1	151-2	EtOH	$C_{30}H_{33}F_2N_7$, 2 $C_4H_4O_4$[e]
30		1	210	EtOH, Et_2O	$C_{31}H_{35}ClN_8O_2S$, 2 HCl, 0.5 H_2O
31		1	202	EtOH	$C_{27}H_{31}N_7$, 1.5 $C_4H_4O_4$[d]
32		1	186	EtOH	$C_{31}H_{35}N_7O$, 2 $C_4H_4O_4$[d], 0.5H_2O
33		1	188	EtOH	$C_{33}H_{39}N_7O$, 2 $C_4H_4O_4$[d], H_2O
34		1	200	EtOH	$C_{32}H_{35}N_7$, 3 $C_4H_4O_4$[d]
35		0	170	EtOH	$C_{31}H_{35}N_7$, $C_4H_4O_4$[d]

[a,d,e]See the corresponding footnotes for Table 1.

Chart 3) does not dramatically change the affinities (compare **28** vs **3**, **30** vs **6**, **31** vs **7**, **32** vs **8**, and **34** vs **10**, respectively).

(2) Part B: The shorter spacers (m = 0) afford compounds with lower affinities for the calcium channel (compare **8** vs **16**, **3** vs **19**, and **6** vs **11** and **12**).

(3) Part C: Modulation of this aromatic part provided the greatest variations in affinities (from 5 nM for **4** to 3700 nM for **17**). When part C is a benzhydryl group, presence of halogens increased affinity (compare **4** vs **5**). Benzo (**21**) or dibenzosuberane groups (**3**) are less favorable than a naphthyl (**7**) group. In the case of a dibenzosuberane-like structure, the nature of the bridge becomes a very sensitive parameter. The affinity decreased as a function of the nature of this bridge: **3** > **8** > **10** ≫ **17**. In the case of **11** and **12**, which are two enantiomers, the (−)-form (**12**) is slightly less active than the (+)-form (**11**).

Biological Results. The study of the MDR-reversing properties of these new derivatives was first carried out in vitro on P388/VCR-20 cells, a murine leukemia cell line whose resistance was induced by vincristine, and KB-A1 cells, a human epidermoid carcinoma cell line whose resistance was induced by adriamycin. The compounds were tested at four concentrations (0.5−5 μM) in association with VCR (P388/VCR-20 cells) or adriamycin (KB-A1 cells). The cytotoxicity due to the modulator alone was systematically measured under the same experimental conditions (four doubling times). Figure 1a shows a typical dose−response curve obtained in KB-A1 cells with VRP and CsA used as reference compounds and compounds **2** and **9** taken as examples. As already shown,[11,12] VRP was moderately active at these relatively low concentrations, and CsA was markedly active only at 5 μM, a toxic concentration (57% of cell viability).

This approach allowed the determination of the most active, noncytotoxic concentration for each modulator. Since the cytotoxicity of these products can be different, we chose, for the purpose of comparison, the two relatively low concentrations of 1 μM for P388/VCR-20 cells and 2.5 μM for KB-A1 cells. Table 5 gives the fold reversal values obtained with these concentrations. Some compounds were nearly inactive (**36**−**38**), and others were significantly more active than S9788 (**9, 28** on P388/VCR-20 cells; **8, 17, 18, 20, 32** on KB-A1 cells), the other ones being approximately as active as S9788.

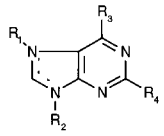
The effect of some selected compounds on ADR accumulation by KB-A1 cells was studied by flow cytometry. Under these experimental conditions (5 h of incubation), all the compounds were devoid of cytotoxicity at 5 μM. Figure 1b shows that **2** and **9** increased ADR accumulation more efficiently than VRP and CsA. This result suggests that these compounds act mainly by inhibiting the Pgp-mediated efflux of cytotoxic drugs, as already discussed for S9788 and derivatives.[13] This is in agreement with the inability of the compounds to increase the sensitivity of the P388 and KB-3-1 cell lines (not shown).

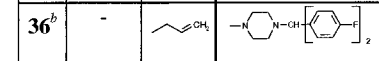
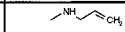
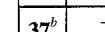
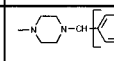
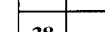
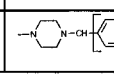
The compounds which were at least as active as S9788 in one cell line in vitro were tested in vivo in association with VCR on the P388/VCR murine leukemia. This leukemia is resistant to VCR, the T/C obtained with 0.25 mg/kg VCR ip (QDX4.1) being on average 141% versus 208% on the sensitive P388. Three doses of tested compounds (25, 50, and 100 mg/kg po) were routinely used in association with 0.25 mg/kg VCR ip. We chose the oral route of administration in order to maintain, as far as possible, plasma levels long enough to fully restore the sensitivity of resistant cells.[12] When tested alone, the compounds were devoid of both toxicity and antitumor activity at 100 mg/kg po (T/C = 91−116%). Table 5 gives the increase in VCR antitumor activity induced by the compounds, expressed as T/V (defined in the Experimental Section). Some compounds significantly increased the survival time of mice when administered in association with VCR with respect to mice treated by VCR alone, the more active ones being **3, 4, 9, 25,** and **26**. For these derivatives, the sensitization was complete, since the T/C obtained in P388/VCR-bearing mice with the association was similar to that obtained in mice bearing the sensitive P388 leukemia with VCR alone.

In order to study the relationship between calcium channel affinity and reversal of MDR, all the compounds were tested for the inhibition of [^{3}H]D888 binding to L-type channel of rat skeletal muscle cell. Table 5 lists the values of K_i obtained in this assay. A large range of affinities were measured, from 5 nM (**4**) to 3700 nM (**17**). The lack of relationship between calcium channel affinity and in vitro reversal of resistance is illustrated in Figure 2.

The mechanism, at the molecular level, of the inhibition of Pgp function remains unknown. However, the modulators are likely to interact directly with Pgp, as shown, for example, by the covalent binding of photoaffinity substrates.[14] The verapamil analog LU-49888 was shown to bind both to Pgp[15] and the α1-subunit of L-type calcium channel,[16] suggesting the existence of homologous sites within these two proteins. We can thus hypothesize similar interactions for our compounds, some being more specific for the calcium channel and others for Pgp, which could explain the lack of

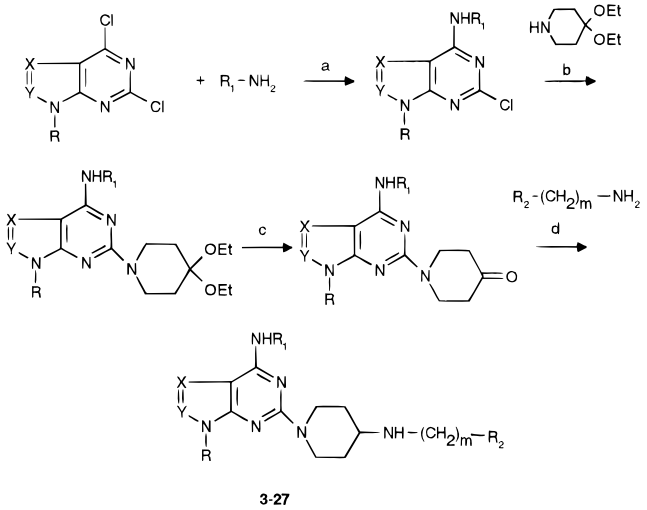
Table 3. Target Compounds: Piperazinopurines



n.	R1	R2	R3	R4	mp,°C	cryst solvent	formula[a]
36[b]	-				170-1	Et_2O	$C_{28}H_{29}F_2N_7$
37[b]	-				208-9	Isopropyl ether	$C_{28}H_{29}F_2N_7$
38		-			159-0	Et_2O	$C_{28}H_{29}F_2N_7$, H_2O

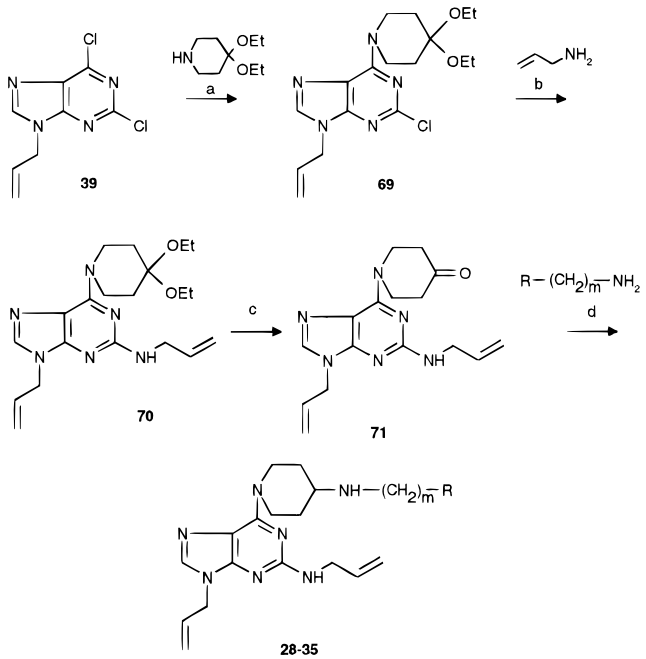
[a,b] See the corresponding footnotes for Table 1.

Scheme 1. Compounds **3**−**27** (Table 1)[a]



[a] Reagents and reaction conditions: (a) 2 equiv of R_1NH_2 in EtOH at room temperature and for 30 min at 50 °C; (b) 10 equiv of 4,4-diethoxypiperidine for 4 h at 100 °C; (c) EtOH, HCl at 50 °C for 2 h; (d) 2 equiv of $NaBH(OAc)_3$ in CH_2Cl_2 at room temperature for 5 h. X, Y, R, R_1, R_2, and *m* have the same meaning as mentioned in Table 1.

Scheme 2. Compounds **28**−**35** (Table 2)[a]



[a] Reagents and reaction conditions: (a) 2 equiv of 4,4-diethoxypiperidine in EtOH for 1 h at room temperature; (b) 10 equiv of allylamine in EtOH, autoclave for 40 h at 100 °C; (c) EtOH, HCl at 50 °C for 2 h; (d) 2 equiv of $NaBH(OAc)_3$ in CH_2Cl_2 at room temperature for 5 h. R and *m* have the same meaning as mentioned in Table 2.

relationship between affinity for the calcium channel and reversal of MDR.

Conclusions

The present work deals with the synthesis and evaluation, in vitro and in vivo, of a series of agents modulating multidrug resistance derived from S9788. During the phase 1 clinical study of S9788, electrocardiographic followup showed a dose-limiting side effect.[2,3] Because of the affinity of S9788 for the calcium channel, we systematically measured the ability of the compounds to inhibit [^{3}H]D888 binding.

Considering the in vitro MDR reversal, the majority of the 36 compounds tested in this work were at least as active as S9788 and markedly more active than verapamil. No relationship between affinity for calcium channel and MDR reversal in vitro was observed. The compounds were tested in vivo on the P388/VCR murine leukemia in association with vincristine. The five more active compounds (**3**, **4**, **9**, **25**, **26**) totally restored the sensitivity of this tumor to VCR when administered po for 4 days. Interestingly, they have different affinities for the phenylalkylamine binding site of the calcium channel, ranging from 5 to 560 nM. Investigations of the cardiovascular side effects of S9788 and these new analogs are now in progress in animal models.

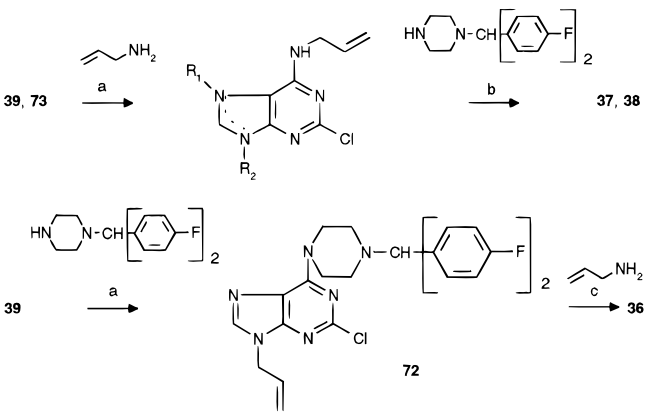
Experimental Section

Biological Procedures. Cell Culture and Cytotoxicity Assay. The reversal agents were solubilized at 10^{-2} M in DMSO (except CsA solubilized in absolute ethanol) and diluted directly in culture medium. The maximum concentration of DMSO used (0.5%) was not cytotoxic and had no effect on chemosensitivity. Verapamil (VRP) was obtained from Sigma Co. Vincristine (VCR) and adriamycin (ADR) were obtained from Roger Bellon (Neuilly, France) and were dissolved in water, and CsA was a generous gift of Sandoz Co.

KB-A1, a human epidermoid carcinoma, was kindly provided by Dr. Gottesman (Bethesda, MD) and is 340-fold more resistant to ADR with respect to the sensitive cell line KB-3-1. P388/VCR-20 was obtained by culturing P388/VCR cells in culture medium containing 20 nM VCR[17] and was 69-fold more resistant to VCR with respect to the corresponding sensitive cell line P388.

These cell lines were previously characterized with respect to Pgp overexpression and cross-resistance to MDR drugs.[13,17]

Scheme 3. Compounds **36**−**38** (Table 3)[a]



[a] Reagents and reaction conditions: (a) 2 equiv of amine in EtOH for 1 h at room temperature and for 30 min at 50 °C; (b) 2 equiv of amine in EtOH for 24 h at 150 °C in an autoclave; (c) 6.5 equiv of allylamine in nBuOH for 72 h at 150 °C in an autoclave. R_1 and R_2 have the same meaning as mentioned in Table 3.

Cells were cultivated in RPMI 1640 medium supplemented with 10% fetal calf serum (Gibco), 2 mM L-glutamine, 100 units/mL penicillin, 100 μg/mL streptomycin, and 10 mM HEPES buffer (pH = 7.4). The cytotoxicity was measured by the microculture tetrazolium assay essentially as previously described.[1] Cells were exposed for four doubling times (48 h for P388 cells, 96 h for KB cells) to both cytotoxic drug (nine graded concentrations in triplicate) and the reversal agent. Results are expressed as IC_{50} values, the drug concentration inhibiting by 50% the absorbance with respect to untreated cells.

The activity of the reversal agents is expressed as fold reversion (FR = IC_{50}(cytotoxic alone)/IC_{50}(cytotoxic + modulator)), calculated for each concentration of modulator agent. To monitor the effect of the modulator agents on cell viability, systematic controls were performed under the same conditions, and the results are expressed as percent viability (absorbance cells grown in the presence of modulator/absorbance control cells) ×100.

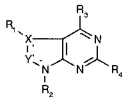
ADR Uptake Studies. KB-A1 cells (5×10^5/mL) were incubated with 50 μM ADR at 37 °C for 5 h with 2.5−10 μM of the tested compounds. For each sample the ADR fluorescence of 10 000 cells was analyzed, at 4 °C, on an ATC3000 flow cytometer (Bruker, Wissembourg, France) using an argon laser (Spectra-Physics, Les Ulis, France) emitting 600 mW at 488 nm. Results are expressed as the increase of the mean of ADR fluorescence of treated cells compared with the mean of ADR fluorescence of untreated cells.

Antitumor Activity. The parental sensitive P388 murine leukemia and P388/VCR, the subline resistant to VCR, were provided by the NCI (Frederick, MD). Groups of 8−10 B6D2F1 female mice (Iffa Credo, Lyon, France) weighing 18−20 g were transplanted ip with 10^6 cells on day 0. The drugs were administrated po daily on days 1−4. The modulator agents were formulated as a suspension in 2.5% Tween 80/H_2O (v/v) and administered 30−60 min before VCR. Antitumor activity was expressed in terms of T/V = (median survival time of modulator + VCR-treated group)/(median survival time of VCR-treated group).

Binding to the Calcium Channels. Rat skeletal muscle microsomes (100 μg/mL) prepared as described[18] were incubated in 20 mM Mops/Na^+ (pH 7.4) containing 1 mM EDTA and 0.1% bovin serum albumin. After a 60 min incubation at 20 °C in the presence of 1 nM [^{3}H]-(−)-D888 with or without tested compounds, the samples were filtered under vacuum on GF/C filters pretreated with 0.1% poly(ethylenimine) and counted by liquid scintillation. Nonspecific binding was determined in a parallel incubation containing an excess of 10 μM (−)-D888. All binding data were analyzed using Lundon 2 (Lundon Software Inc., Cleveland).

General Methods for Preparation of Compounds. All nonaqueous reactions were carried out under an atmosphere of nitrogen. Flash column chromatography was carried out

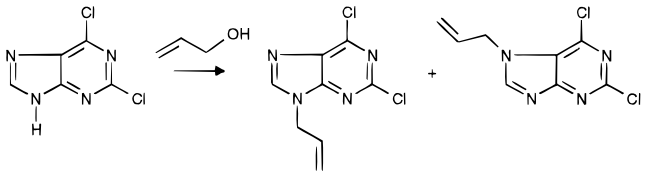
Table 4. Starting Materials



n.	R1	R2	R3	R4	X	Y	mp,°C	formula[a]
39[b]	-		Cl	Cl	N	C	73-4	$C_8H_6Cl_2N_4$
40	-			Cl	N	C	110-2	$C_{11}H_{12}ClN_5$
41	-				N	C	118	$C_{20}H_{30}N_6O_2$
42	-				N	C	138	$C_{16}H_{20}N_6O$
43[b]	-		Cl	Cl	C	N	60-3	$C_8H_6Cl_2N_4$
44	-			Cl	C	N	115-9	$C_{11}H_{12}ClN_5$
45	-				C	N	88-2	$C_{20}H_{30}N_6O_2$
46	-				C	N	93-5	$C_{16}H_{20}N_6O$
47	-		Cl	Cl	C	C	64-8	$C_9H_7Cl_2N_3$
48	-			Cl	C	C	62-5	$C_{12}H_{13}ClN_4$
49	-				C	C	oil	$C_{21}H_{31}N_5O_2$
50	-				C	C	70-3	$C_{17}H_{21}N_5O$
51	-			Cl	C	N	112-4	$C_{11}H_{14}ClN_5$
52	-				C	N	126	$C_{20}H_{32}N_6O_2$
53	-				C	N	135	$C_{16}H_{22}N_6O$
54	-		Cl	Cl	N	C	58	$C_8H_8Cl_2N_4$
55	-			Cl	N	C	110	$C_{11}H_{14}ClN_5$
56	-				N	C	135	$C_{20}H_{32}N_6O_2$
57	-				N	C	135	$C_{16}H_{22}N_6O$
58[b]	-			Cl	N	C	117	$C_{11}H_{14}ClN_5$
59[b]	-				N	C	117	$C_{20}H_{32}N_6O_2$
60[b]	-				N	C	122	$C_{16}H_{22}N_6O$
61	-			Cl	N	C	117-9	$C_{11}H_{16}ClN_5$
62	-				N	C	113-5	$C_{20}H_{34}N_6O_2$
63	-				N	C	138-9	$C_{16}H_{24}N_6O$
64[b]	-		OH	OH	N	N	281	$C_7H_7N_5O_2$
65[b]	-		Cl	Cl	N	N	59	$C_7H_5Cl_2N_5$
66	-			Cl	N	N	-	not isolated
67	-				N	N	185-8	$C_{19}H_{29}N_7O_2$
68	-				N	N	160-2	$C_{15}H_{19}N_7O$
69[b]	-			Cl	N	C	oil	$C_{17}H_{24}ClN_5O_2$
70[b]	-				N	C	oil	$C_{20}H_{30}N_6O_2$
71[b]	-				N	C	97	$C_{16}H_{20}N_6O$
72[b]	-			Cl	N	C	162	$C_{25}H_{23}ClF_2N_6$
73[b]		-	Cl	Cl	N	C	102-3	$C_8H_6Cl_2N_4$
74		-		Cl	N	C	114-6	$C_{11}H_{12}ClN_5$

[a] C, H, N analyses were within 0.4% of theoretical values for the formulae given, except for **43**: N calcd, 30.95; found, 30.54. All compounds exhibited NMR consistent with assigned structures. [b] Synthesis described in the Experimental Section.

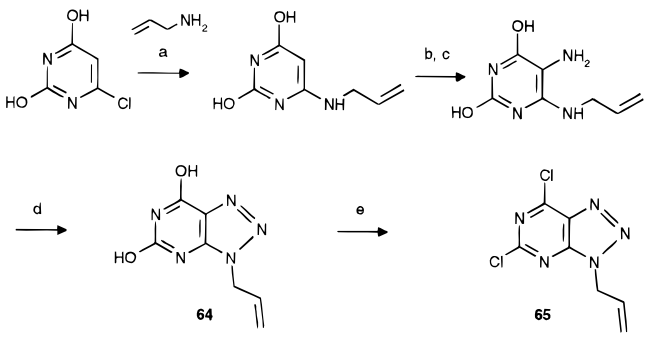
Scheme 4. Compounds **39** and **73** (Table 4)[a]



39 + **73**

[a] Reagents and reaction conditions: 1.5 equiv of $P(C_6H_5)_3$, 2 equiv of allyllic alcohol, 1.5 equiv of DEAD in THF overnight at room temperature.

Scheme 5. Compounds **64** and **65** (Table 4)[a]



64 **65**

[a] Reagents and reaction conditions: (a) excess of allylamine for 4 h at 120 °C; (b) 1.1 equiv of $NaNO_2$ in H_2O−AcOH for 45 min at 50 °C; (c) 3.4 equiv of $Na_2S_2O_4$ in H_2O for 15 min at 80 °C; (d) $NaNO_2$ in H_2O, HCl (4 N) up to pH 5; (e) $C_6H_5POCl_2$ in excess for 24 h at 160 °C.

with Amicon silica gel (230−400 mesh) under medium nitrogen pressure. ^{1}H NMR were recorded on either a Bruker AC200 or a Bruker AM400 at 200 and 400 MHz, respectively. ^{13}C NMR were measured at 50.29 or 75.43 MHz. Chemical shifts are expressed in ppm downfield from internal tetramethylsilane. Significant ^{1}H NMR data are reported in the following order: multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet), number of protons. IR spectra were recorded on a Bruker IFS48 infrared spectrometer. Elemental analyses were carried out on a Carlo ERBA autoanalyzer. All melting points were determinated on a MEL TEMP capillary apparatus and are uncorrected.

Starting Materials: For the Preparation of Compounds 3−38 (Tables 1−3). 2,2-Diphenylethylamine, 1-naphthalenemethylamine, and 1-[bis(4-fluorophenyl)methyl]piperazine are commercially available. (10,11-Dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-yl)methylamine, (8-chloro-10,10-dioxo-11-methyl-5,11-dihydrodibenzo[*c*,*f*][1,2]thiazepin-5-yl)methylamine, (6,11-dihydrodibenz[*b*,*e*]oxepin-11-yl)methylamine, (2-methoxy-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-yl)-methylamine, (5*H*-dibenzo[*a*,*d*]cyclohepten-5-yl)methylamine, (6-oxo-5,11-dihydro-6*H*-dibenz[*b*,*e*]azepin-11-yl)methylamine, 5-amino-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cycloheptene, and 2,2,2-triphenylethylamine have been described in a preceding paper.[1] [Bis(4-fluorophenyl)methyl]amine,[19] (+)-5-amino-8-chloro-10,10-dioxo-11-methyl-5,11-dihydrodibenzo[*c*,*f*][1,2]thiazepine,[20] 11-amino-6,11-dihydrodibenz[*b*,*e*]oxepine,[21] and 1-(aminomethyl)benzosuberane[22] were prepared by reported procedures. 5-Chloro-10,10-dioxo-11-propyl-5,11-dihydrodibenzo[*c*,*f*][1,2]thiazepine[23] was substituted with AgCN in CH_3CN for 2 h under reflux to give the corresponding nitrile (mp 102−103 °C, 46%), which was reduced by BMS in THF for 2 h under reflux to give 5-(aminomethyl)-10,10-dioxo-11-propyl-5,11-dihydrodibenzo[*c*,*f*][1,2]thiazepine (oil, 70%): IR (neat) 3383−3323 (NH_2), 1329−1149 cm^{-1} (SO_2); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.9 (d, 1H, C*H*═), 7.45−7.2 (m, 7H, C*H*═), 4.1 (t, 1H, CH_2-C*H*-C), 3.8−3.45 (m, 2H, N-CH_2-CH_2), 3.3 (d, 2H, CH-CH_2-NH_2), 1.7 (m, 2H, CH_2-CH_2-CH_3), 1.1 (bd, 2H, NH_2, exchangeable for D_2O), 0.85 (t, 3H, CH_2-CH_3). Anal. ($C_{17}H_{20}N_2O_2S$) C, H, N, S.

(2,3,4-Trimethoxy-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-yl)methylamine. This was made by analogy with the reported 2-methoxy homolog.[24] Phthalic anhydride, trimethoxybenzoic acid, and sodium acetate were heated for 3 h at 240 °C. The resulting (trimethoxybenzylidene)phthalide (162 °C, 68%) was hydrogenated for 24 h at 150 °C and 150 kg of H_2 pressure to give the 2-(methoxyphenethyl)benzoic acid (166 °C, 33%). The latter was cyclized by means of P_2O_5 in xylene under reflux for 1 h to give the corresponding trimethoxydibenzosuberone (oil, 31%). The ketone was reduced in alcohol with $LiAlH_4$ in Et_2O for 2 h under reflux (mp 57−60 °C, 50%). The alcohol was chlorinated with HCl gas in CH_2Cl_2 at 10 °C for 2 h (131 °C, 100%). The chloride was substituted with AgCN in CH_3CN for 3 h under reflux to give the corresponding nitrile (mp 111 °C, 42%) which was reduced by means of BMS in THF under reflux for 3 h to give the title product (oil, 58%): IR (neat) 2850 cm^{-1} (NH_2); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.7 and 7.1 (2m, 4H, C*H*═), 6.4 (s, 1H, C*H*═), 4.35 (dd, 1H, C*H*-CH_2-NH_2), 3.8 (3s, 9H, 3 × OCH_3), 3.3 (m, 2H, CH_2-CH_2), 3.1 (dd, 2H, CH-CH_2-NH_2), 2.8 (m, 2H, CH_2-CH_2), 1.4 (bd, 2H,-NH_2, exchangeable for D_2O).

(−)-5-Amino-8-chloro-10,10-dioxo-11-methyl-5,11-dihydrodibenzo[*c*,*f*][1,2]thiazepine: prepared by analogy with the reported (+)-isomer[20] and using (+)-dibenzoyltartaric acid.

For the Preparation of Compounds 39−74 (Table 4). 2,6-Dichloropurine is a commercial product. 4,6-Dichloro-1*H*-pyrazolo[3,4-*d*]pyrimidine[10] and 2,4-dichloro-1*H*-pyrrolo[2,3-*d*]pyrimidine[9] were prepared as reported.

9-Allyl-2,6-dichloropurine (39) and 7-Allyl-2,6-dichloropurine (73). Diethyl azodicarboxylate (67.9 g, 0.39 mol) was added dropwise over 30 min at room temperature to a stirred solution of 2,6-dichloropurine (50 g, 0.26 mol), triphenylphosphine (102.3 g, 0.39 mol), and allylic alcohol (35.4 mL, 0.52 mol) in THF (1 L). The temperature increased to 50 °C. The mixture was stirred overnight at room temperature and evaporated to dryness. The crude residue was poured into Et_2O, and the insoluble part was filtered off. The filtrate was concentrated, and the residue was purified by flash chromatography eluting with CH_2Cl_2−acetone (95:5) to provide 43.7 g (73%) of **39**: mp 73−74 °C; IR (Nujol) 1620−1593 cm^{-1} (C═C, C═N); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.1 (s, 1H, N═C*H*-N), 6.2−5.9 (m, 1H, CH_2-C*H*═CH_2), 5.5−5.3 (m, 2H, CH═CH_2), 4.85 (m, 2H, CH_2−CH═). Anal. ($C_8H_6Cl_2N_4$) C, H, N, Cl.

Further eluting provided 11 g (18.5%) of **73**: mp 102−103 °C; IR (Nujol) 1640 cm^{-1} (C═C); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.3 (s, 1H, N═C*H*-N), 6.2−6.0 (m, 1H, CH_2-C*H*═CH_2), 5.45−5.2 (m, 2H, CH═CH_2), 5.15 (d, 2H, CH_2-CH═). Anal. ($C_8H_6Cl_2N_4$) C, H, N, Cl.

1-Allyl-4,6-dichloropyrazolo[3,4-*d*]pyrimidine (43). A representative procedure is as follows for the conversion of **47** (90%). 4,6-Dichloropyrazolo[3,4-*d*]pyrimidine[10] (38.1 g, 0.20 mol), triphenylphosphine (76 g, 0.3 mol), and allylic alcohol (29 mL, 0.42 mol) were stirred in THF while diethyl azodicarboxylate (48 mL, 0.3 mol) was added dropwise over 30 min at room temperature. The solvent was concentrated, and the crude residue was dissolved in Et_2O. The insoluble materials were filtered off, and the resulting solution was evaporated under vacuum. The residue was purified by flash chromatography eluting with CH_2Cl_2 to provide **43** which crystallized on standing (11.4 g, 24.9%): mp 60−63 °C; IR (Nujol) 1645, 1589 cm^{-1} (C═N, C═C); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.15 (s, 1H, N═C*H*), 6.2−5.9 (m, 1H, CH_2-C*H*═CH_2), 5.3 (m, 2H, CH═CH_2), 5.05 (d, 2H, N-CH_2). Anal. ($C_8H_6Cl_2N_4$) C, H, N, Cl.

47: mp 64−68 °C; IR (Nujol) 1643 (C═C), 1589 cm^{-1} (C═N); ^{1}H NMR (DMSO-d_6, 200 MHz) δ 7.1 (d, 1H, N-C*H*═), 6.7 (d, 1H, N-CH═C*H*), 6.0 (m, 1H, CH_2-C*H*═), 5.2−4.9 (m, 2H, ═CH_2), 4.85 (d, 2H, CH_2). Anal. ($C_9H_7Cl_2N_3$) C, H, N, Cl.

9-Allyl-2-chloro-6-(propylamino)purine (58). A representative procedure is as follows for the conversions of **39** → **40** (72.2%), **43** → **44** (96%), **47** → **48** (92%), **43** → **51** (71%), **54** → **55** (90%), **54** → **61** (80.2%). A solution of **39** (40 g, 0.175 mol) and *n*-propylamine (20.7 g, 0.35 mol) in EtOH (700 mL) was stirred for 1 h at room temperature and then for 30 min at 50 °C. EtOH was evaporated in vacuo and the residue treated with CH_2Cl_2 and water. The organic layer was separated and dried ($MgSO_4$), and the solvent was evaporated

Table 5. Pharmacological Properties of the Target Compounds

compd	calcium channel affinity[a] K_i [^{3}H]D888, nM	in vitro reversal fold reversion[b] (% of viability)[c] P388/VCR-20	KB-A1	in vivo reversal T/V[d] (optimal dose po, mg/kg)
2	89	42 ± 7 (93 ± 3)	189 ± 28 (111 ± 4)	1.19 (200)
VRP	58	4 (91)	14 (79 ± 6)	1.22 (50)
CsA	ND	260 (65)	9 (82)	ND
3	46	50 (98)	171 (104)	1.42 (100)
4	5	59 (95)	121 (120)	1.49 (100)
5	630	78 (93)	278 (115)	1.30 (100)
6	87	75 (70)	238 (121)	1.13 (100)
7	440	53 (80)	236 (110)	1.33 (100)
8	120	84 (95)	406 (108)	1.36 (100)
9	12	236 (114)	160 (108)	1.54 (100)
10	170	93 (111)	208 (110)	1.21 (50)
11	650	124 (146)	102 (60)	1.27 (100)
12	1000	57 (135)	68 (64)	1.11 (100)
13	270	30 (74)	120 (132)	1.31 (100)
14	490	57 (96)	75 (145)	1.21 (100)
15	99	108 (96)	136 (86)	1.16 (100)
16	550	37 (123)	44 (109)	ND
17	3700	71 (106)	499 (126)	1.13 (100)
18	200	108 (126)	723 (86)	1.24 (100)
19	770	15 (111)	83 (111)	ND
20	410	27 (119)	370 (127)	1.30 (100)
21	62	78 (123)	272 (122)	1.20 (50)
22	420	56 (98)	147 (86)	1.30 (100)
23	400	75 (90)	152 (103)	1.18 (100)
24	48	51 (102)	209 (107)	1.31 (100)
25	84	70 (103)	171 (112)	1.38 (50)
26	560	129 (98)	156 (127)	1.57 (100)
27	860	13 (83)	264 (84)	1.17 (100)
28	62	288 (112)	210 (95)	1.19 (100)
29	ND	36 (108)	49 (106)	ND
30	170	70 (89)	214 (141)	1.03 (100)
31	1600	35 (104)	113 (106)	1.31 (100)
32	93	190 (91)	578 (110)	1.19 (100)
33	110	133 (131)	200 (91)	1.06 (100)
34	90	193 (119)	189 (102)	1.17 (100)
35	620	24 (120)	142 (119)	ND
36	ND	13 (98)	6 (73)	ND
37	ND	24 (99)	9 (103)	ND
38	ND	3 (97)	5 (104)	ND

[a] K_i inhibition constant of [^{3}H]D888 binding to calcium channel of membranes from rat skeletal muscle. [b] Ratio of IC_{50}(cytotoxic alone (VCR for P388/VCR-20, ADR for KB-A1 cells))/IC_{50}(cytotoxic + modulator) (1 μM in association with VCR or 2.5 μM in association with ADR). [c] Percent of cell viability remaining after incuation with the modulator alone. [d] Ratio of the survival time of mice treated with VCR (0.25 mg/kg ip) + modulator given at the optimal dose po/survival time of mice treated with VCR alone. Modulators were adminstered 60 min before VCR, on days 1−4 after tumor implantation. ND: not done.

to provide 37.4 g (84.9%) of **58** as yellowish gray crystals: mp 117 °C; IR (Nujol) 3271 (NH), 1618 cm^{-1} (C═C, C═N); ^{1}H NMR (DMSO-d_6, 200 MHz) δ 8.3 (m, 1H, N*H*, exchangeable for D_2O), 8.1 (s, 1H, N═C*H*-N), 6.2−6.0 (m, 1H, CH_2-C*H*═CH_2), 5.25−5.0 (m, 2H, CH═CH_2), 4.75 (d, 2H, N-CH_2-CH═), 3.4 (m, t after exchange, 2H, NH-CH_2-CH_2), 1.6 (m, 2H, CH_2-CH_2-CH_3), 0.85 (t, 3H, CH_3). Anal. ($C_{11}H_{14}ClN_5$) C, H, N, Cl.

9-Allyl-2-(4,4-diethoxypiperidin-1-yl)-6-(propylamino)-purine (59). A representative procedure is as follows for the conversions of **40** → **41** (88%), **44** → **45** (93.3%), **48** → **49** (30%), **51** → **52** (82.6%), **55** → **56** (87%), **61** → **62** (68.5%), **66** → **67** (60%). **58** (25 g, 0.099 mol) and 4,4-diethoxypiperidine (175 g, 1.01 mol) were stirred for 4 h at 100 °C. Excess 4,4-diethoxypiperidine was evaporated in vacuo. The crude residue was treated with CH_2Cl_2 and water. The organic layer was dried and evaporated in vacuo, and the residue was purified by flash chromatography eluting with CH_2Cl_2−acetone (90:10) to provide 31.6 g (82.1%) of **59** as white crystals: mp 117 °C; ^{1}H NMR (DMSO-d_6, 200 MHz) δ 7.7 (s, 1H, N═C*H*-N), 7.3 (m, 1H, N*H*, exchangeable for D_2O), 6.0 (m, 1H, CH_2-C*H*═CH_2), 5.2−5.0 (m, 2H, CH═CH_2), 4.6 (d, 2H, N-CH_2-CH═), 3.7 (m, 4H, NH-CH_2-, C*H*-N-C*H*), 3.45 (m, 6H, 2 × O-CH_2-CH_3, C*H*-N-C*H*), 1.8 (m, 6H, CH_2-CH_2-CH_3, 2 × CH_2-CH_2-N), 1.1 (t, 6H, 2 × CH_3-CH_2-O), 0.85 (t, 3H, CH_2-CH_2-CH_3). Anal. ($C_{20}H_{32}N_6O_2$) C, H, N.

9-Allyl-2-(4-oxopiperidin-1-yl)-6-(propylamino)purine (60). A representative procedure is as follows for the conversions of **41** → **42** (98%), **45** → **46** (90%), **49** → **50** (89%), **52** → **53** (88%), **56** → **57** (98%), **62** → **63** (91%), **67** → **68** (97.5%). A solution of **59** (30 g, 0.077 mol) in EtOH (250 mL) and HCl (250 mL) was stirred at 50 °C for 2 h. EtOH was evaporated in vacuo, the crude residue was treated with CH_2Cl_2 and 10% Na_2CO_3 solution, and the organic layer was separated and washed with water. After drying, the solvent was evaporated in vacuo to provide 22 g (90.6%) of yellowish gray crystalline residue **60**: mp 122 °C; IR (Nujol) 3271−3207 (NH), 1712 (C═O), 1628−1600 cm^{-1} (C═C, C═N); ^{1}H NMR (DMSO-d_6, 200 MHz) δ 7.75 (s, 1H, N═C*H*-N), 7.45 (m, 1H, N*H*, exchangeable for D_2O), 6.2−6.0 (m, 1H, CH_2-C*H*═CH_2), 5.3−5.0 (m, 2H, CH═CH_2), 4.65 (d, 2H, N-CH_2-CH═), 4.0 (t, 4H, CH_2-N-CH_2), 3.4 (m, 2H, NH-CH_2-), 2.35 (t, 4H, CH_2-O-CH_2), 1.6 (m, 2H, CH_2-CH_2-CH_3), 0.9 (t, 3H, CH_3). Anal. ($C_{16}H_{22}N_6O$) C, H, N.

3-Allyl-5,7-dihydroxy-1,2,3-triazolo[4,5-*d*]pyrimidine (64). 6-Chlorouracil (25.5 g, 0.174 mol) and allylamine (200 mL) were stirred for 4 h at 120 °C. After cooling, the mixture was filtered, and the solid was washed with Et_2O and dissolved in 150 mL of NaOH (2 N). The solid residue was filtered off, and the solution was cautiously acidified with concentrated AcOH (pH ∼6) and then cooled. The precipitate was filtered and washed with water and Et_2O to provide 25 g (85.9%) of 6-(allylamino)uracil: mp 245−250 °C (lit.[25] mp 254−255 °C). A mixture of 6-(allylamino)uracil (20 g, 0.12 mol) with $NaNO_2$ (10 g, 0.14 mol) in H_2O (200mL) was heated at 50 °C. AcOH was added to achieve pH 4.5, the mixture was stirred for 45 min at 50 °C and cooled, and the resulting precipitate was filtered and washed with H_2O and Et_2O to provide 12 g (51%) of 6-(allylamino)-5-nitrosouracil: mp 218 °C.

This compound was dissolved in water (20 mL) at 80 °C, and $Na_2S_2O_4$ (3 g, 0.015 mol) was added portionwise with

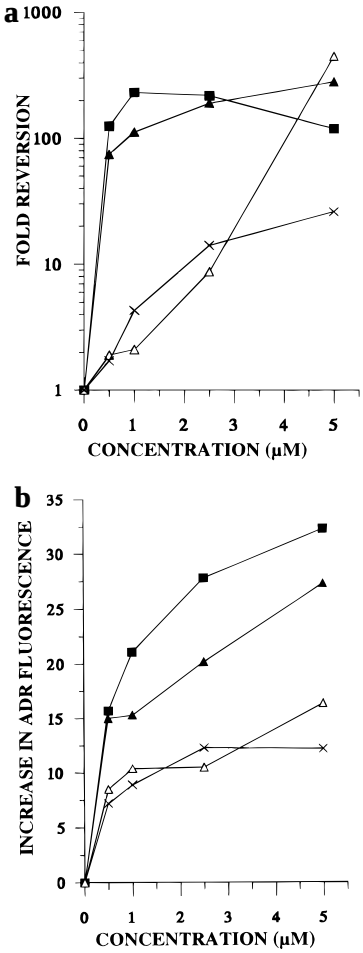


Figure 1. In vitro activity of compounds **2** (▲) and **9** (■) on KB-A1 cells compared with VRP (×) and CsA (△). (a) Cells were exposed for 4 days to ADR alone or to ADR + modulator, and the IC_{50} values were mesured by the microculture tetrazolium assay. Fold reversion is the ratio of the IC_{50}(ADR alone)/IC_{50}(ADR + modulator). (b) Cells were incubated with 50 μM ADR for 5 h with ADR alone or ADR + modulator. The increase in ADR uptake is (mean of ADR fluorescence of treated cells) - (mean of ADR fluorescence of untreated cells).

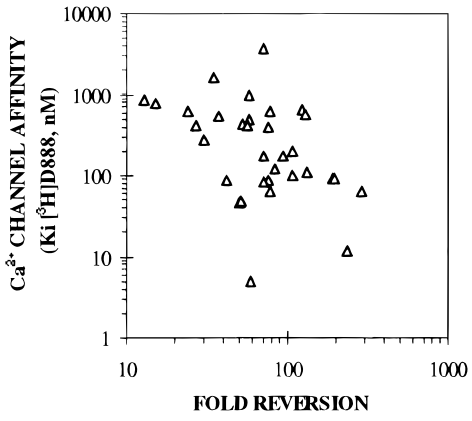


Figure 2. Lack of direct relationship between calcium channel affinity and sensitization of KB-A1 to ADR.

stirring until discoloration and precipitation. Stirring was continued for 15 min at 80 °C, and then the mixture was cooled to 0 °C and filtered. The crude solid obtained was suspended in water (20 mL) containing 1.3 g of $NaNO_2$. A 4 N HCl solution was then added dropwise up to pH 5 at 5−10 °C. Stirring was continued for 10 min at 10 °C and for 1 h at room temperature. After standing on cooling, the precipitate was filtered, washed with water, and dried to provide 1 g (30%) of 3-allyl-5,7-dihydroxy-1,2,3-triazolo[4,5-*d*]pyrimidine (**64**): mp 281 °C; IR (Nujol) 3400−2500 (OH + H_2O), 1741−1700 (C=N), 1630 cm^{-1} (C=C); ^{1}H NMR (DMSO-d_6, 200 MHz) δ 12.5−12.0 (m, 1H, O*H*, exchangeable for D_2O), 11.25 (m, 1H, O*H*, exchangeable for D_2O), 6.0 (m, 1H, C*H*=CH_2), 5.3−4.9 (m, 4H, CH_2-CH=CH_2); ^{13}C NMR (DMSO-d_6, 75.7 MHz) δ 48.8, 118.3, 124.2, 131.6, 141.7, 150.6, 156.2. Anal. ($C_7H_7N_5O_2$) C, H, N.

3-Allyl-5,7-dichloro-1,2,3-triazolo[4,5-*d*]pyrimidine (65). A mixture of **64** (8.7 g, 0.045 mol) and phenylphosphine dichloride (25 mL) was stirred for 24 h at 160 °C. This mixture was cooled, triturated with crushed ice, and partitioned between water and CH_2Cl_2. The organic layer was separated, washed with water, dried ($MgSO_4$), and concentrated. The crude material was purified by flash chromatography, eluting with CH_2Cl_2, and then triturated with isopropyl ether to provide 5.7 g (55%) of **65**: mp 59 °C; IR (Nujol) 1647 (C=C), 1581 cm^{-1} (C=N); ^{1}H NMR (DMSO-d_6, 200 MHz) 6.2−6.0 (m, 1H, C*H*=CH_2), 5.4−5.1 (m, 4H, CH_2-CH=CH_2). Anal. ($C_7H_5Cl_2N_5$) C, H, N, Cl.

9-Allyl-2-chloro-6-(4,4-diethoxypiperidin-1-yl)purine (69). A solution of 4,4-diethoxypiperidine (80.7 g, 0.46 mol) and 9-allyl-2,6-dichloropurine (**39**) (53.5 g, 0.23 mol) in EtOH (1.3 L) was stirred for 1 h at room temperature and then concentrated. The crude material was partitioned between CH_2Cl_2 and water; the organic layer was separated, washed in water, dried ($MgSO_4$), and concentrated to provide 71.5 g of **69** (85%) as an oil: IR (neat) 1645 cm^{-1} (C=C); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.65 (s, 1H, N=C*H*-N), 6.15−5.9 (m, 1H, C*H*=CH_2), 5.35 (m, 2H, CH=CH_2), 4.75 (d, 2H, N-CH_2-CH=), 4.6−3.8 (m, 4H, CH_2-N-CH_2), 3.5 (q, 4H, 2 × OCH_2), 1.9 (m, 4H, CH_2-CH-N-CH-CH_2), 1.25 (t, 6H, 2 × CH_2-CH_3). Anal. ($C_{17}H_{24}ClN_5O_2$) C, H, N, Cl.

9-Allyl-2-(allylamino)-6-(4,4-diethoxypiperidin-1-yl)purine (70). A solution of **69** (1 g, 0.0027 mol), allylamine (2 mL, 0.027 mol), KI (1 crystal), and EtOH (6 mL) was heated in an autoclave for 40 h at 100 °C. The mixture was concentrated, the resulting residue was treated with CH_2Cl_2 and water, and the organic layer was separated, dried ($MgSO_4$), and concentrated. The crude product was purified by flash chromatography eluting with CH_2Cl_2−acetone (95:5) to provide 0.72 g (69%) of **70** as an oil: IR (neat) 3363 (NH), 1645−1593 cm^{-1} (C=C, C=N); ^{1}H NMR (DMSO-d_6, 200 MHz) δ 7.7 (s, 1H, N=C*H*-N), 6.5 (t, 1H, N*H*, exchangeable for D_2O), 6.15−5.7 (m, 2H, 2 × C*H*=CH_2), 5.2−4.95 (m, 4H, 2 × =CH_2), 4.6 (d, 2H, N-CH_2-CH=), 4.15 (m, 4H, CH_2-N-CH_2), 3.9 (t, 2H, NH-CH_2-CH=), 3.45 (q, 4H, 2 × OCH_2), 1.7 (m, 4H, CH_2-CH$_2$-N-CH$_2$-CH_2), 1.1 (t, 6H, 2 × CH_3-CH$_2$-O). Anal. ($C_{20}H_{30}N_6O_2$) C, H, N.

9-Allyl-2-(allylamino)-6-(4-oxopiperidin-1-yl)purine (71). A solution of **70** (24.2 g, 0.062 mol), 1 N HCl (240 mL), and ethyl alcohol (240 mL) was heated for 2 h at 50 °C with stirring. The solvent was evaporated and the residue partitioned between CH_2Cl_2 and 10% aqueous Na_2CO_3. The organic layer was separated, washed with water, dried over $MgSO_4$, and condensed to give 18 g (93%) of an oil which crystallized on standing: mp 97 °C; IR (Nujol) 3350 (NH), 1697 (C=O), 1645 cm^{-1} (C=C); ^{1}H NMR (DMSO-d_6, 200 MHz) δ 7.75 (s, 1H, N=C*H*-N), 6.65 (t, 1H, N*H*, exchangeable for D_2O), 6.15−5.75 (m, 2H, 2 × CH_2-C*H*=), 5.25−4.9 (m, 4H, 2 × CH_2=CH), 4.65 (d, 2H, N-CH_2-CH=), 4.4 (m, 4H, CH_2-N-CH_2), 3.85 (m, 2H, NH-CH_2-CH=), 2.45 (m, 4H, CH_2-CO-CH_2). Anal. ($C_{16}H_{20}N_6O$) C, H, N.

9-Allyl-6-[4-[bis(4-fluorophenyl)methyl]piperazin-1-yl]-2-chloropurine (72). A solution of **39** (3.2 g, 0.014 mol) and [bis(4-fluorophenyl)methyl]piperazine in ethyl alcohol (50 mL) was stirred for 1 h at room temperature. Stirring was then continued for 1 h at 50 °C. The solvent was evaporated in vacuo, and the residue was partitioned between CH_2Cl_2 and 10% aqueous Na_2CO_3. The organic layer was separated, washed with water, dried ($MgSO_4$), and concentrated. The residual oil was purified by use of flash chromatography eluting with CH_2Cl_2−acetone (80:20) to provide the crude product which was crystallized from ethyl alcohol to give 5.4 g (80.2%) of the title product **72**: mp 162 °C; IR (Nujol) 1597 cm^{-1} (C=C); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.6 (s, 1H, N=C*H*-N), 7.35 (m, 4H, aromatic *H*), 7.0 (m, 4H,

aromatic *H*), 6.0 (m, 1H, CH_2-C*H*═), 5.25 (m, 2H, CH═CH_2), 4.75 (m, 2H, N-CH_2-CH═), 4.25 (m, 4H, CH_2-N-CH_2), 4.3 (s, 1H, N-C*H*), 2.45 (m, 4H, CH_2-N-CH_2). Anal. ($C_{25}H_{23}ClF_2N_6$) C, H, N, Cl.

Target Compounds (Tables 1−3): 9-Allyl-6-(allylamino)-2-[4-[[(10,11-dihydro-5*H*-dibenzo[*a,d*]cyclohepten-5-yl)-methyl]amino]piperidin-1-yl]purine (3). A representative procedure is provided for the following conversions, and the system of elution employed for flash chromatography is given in parentheses: **42** → **4** (60.7%) (CH_2Cl_2−acetone, 80:20), **42** → **5** (59%) (CH_2Cl_2−MeOH, 96:4), **42** → **6** (33.9%) (CH_2Cl_2−MeOH, 97:3), **42** → **7** (57%) (CH_2Cl_2−MeOH, 97:3), **42** → **8** (89%) (toluene−CH_2Cl_2−MeOH, 50:45:5), **42** → **9** (86%), **42**→ **10** (93.6%), **42** → **11** (80.6%) (toluene−CH_2Cl_2−MeOH, 50:45:5), **42** → **12** (76%) (toluene−CH_2Cl_2−MeOH, 50:45:5), **46** → **13** (97.4%) (CH_2Cl_2−acetone, 80:20), **42** → **14** (81%) (CH_2Cl_2−acetone, 70:30), **42** → **15** (55.8%) (CH_2Cl_2−MeOH, 95:5), **42** → **16** (43%) (CH_2Cl_2−acetone, 80:20), **42** → **17** (64.1%) (CH_2Cl_2−MeOH, 95:5), **50** → **18** (95%) (toluene−MeOH, 99:1), **42** → **19** (66%) (CH_2Cl_2−acetone, 90:10), **42** → **20** (88.9%) (toluene−MeOH, 96:4), **42** → **21** (68%) (CH_2Cl_2−acetone, 70:30), **53** → **22** (58%) (CH_2Cl_2−MeOH, 95:5), **46** → **23** (61.5%) (CH_2Cl_2−acetone, 90:10), **57** → **24** (82%) (toluene−MeOH, 95:5), **60** → **25** (62%) (CH_2Cl_2−acetone, 70:30), **63** → **26** (34%) (CH_2Cl_2−acetone, 70:30), **68** → **27** (62%) (toluene−EtOH, 97:3), **71** → **28** (68, 7%) (CH_2Cl_2−MeOH, 97:3), **71** → **29** (59%) (CH_2Cl_2−acetone, 85:15), **71** → **30** (66%) (toluene−CH_2Cl_2−MeOH, 50:45:5), **71** → **31** (78%) (toluene−CH_2Cl_2−MeOH, 50:45:5), **71** → **32** (84.6%) (CH_2Cl_2−MeOH, 95:5), **71** → **33** (64.4%), **71** → **34** (84%), **71** → **35** (85%) (CH_2Cl_2−acetone, 90:10). A solution of **42** (5 g, 0.016 mol), (10,11-dihydro-5*H*-dibenzo[*a,d*]cyclohepten-5-yl)methylamine (3.6 g, 0.016 mol), and sodium triacetoxyborohydride (7.4 g, 0.035 mol) in CH_2Cl_2 (150 mL) was stirred for 5 h at room temperature. A 10% aqueous Na_2CO_3 solution was added, and the organic layer was separated, washed with water, dried over $MgSO_4$, and concentrated. The resulting oil was purified by flash chromatography eluting with CH_2Cl_2−acetone (80:20) to provide the title product **3** (7.3 g, 87.9%): mp 142−144 °C; IR (Nujol) 3282 (NH), 1610 cm^{-1} (C═N); ^1H NMR (DMSO-d_6, 200 MHz) δ 7.7 (s, 1H, N═C*H*-N), 7.5 (s, 1H, N*H*, exchangeable for D_2O), 7.2 (m, 2H, aromatic *H*), 7.1 (m, 6H, aromatic *H*), 5.95 (m, 2H, 2 × CH_2-C*H*═), 5.1 (m, 4H, 2 × CH═CH_2), 4.6 (d, 2H, N-CH_2-CH═), 4.5 (d, 2H, CH_2-N-CH_2), 4.25 (t, 1H, NH-CH_2-C*H*), 4.1 (s br, 2H, NH-CH_2-CH═), 3.2 (m, 4H, CH_2-CH_2), 3.0 (m, 2H, NH-CH_2-CH), 2.9 (t, 2H, CH_2-N-CH_2), 2.7 (m, 1H, C*H*-NH), 1.8 (m, 2H, CH_2-CH-CH_2), 1.2 (m, 2H, CH_2-CH-CH_2), 1.2 (s br, 1H, N*H*, exchangeable for D_2O). Anal. ($C_{32}H_{37}N_7$) C, H, N.

9-Allyl-2-(allylamino)-6-[4-[bis(4-fluorophenyl)methyl]-piperazin-1-yl]purine (36). A solution of **72** (3 g, 0.0062 mol), allylamine (3 mL, 0.04 mol), potassium iodide (0.05 g), and *n*-butyl alcohol (60 mL) was heated for 72 h at 150 °C in an autoclave. The mixture was cooled, and the solvent was evaporated. The residue was partitioned between CH_2Cl_2 and water. The organic layer was separated, dried over $MgSO_4$, and concentrated. The oily residue was purified by flash chromatography eluting with CH_2Cl_2−acetone (95:5) to provide 0.96 g (31%) of **36**: mp 170−171 °C; IR (Nujol) 3307 (NH), 1645 cm^{-1} (C═C); ^1H NMR (DMSO-d_6, 200 MHz) δ 7.7 (s, 1H, N═C*H*-N), 7.4 (m, 4H, aromatic *H*), 7.15 (m, 4H, aromatic *H*), 6.5 (t, 1H, N*H*, exchangeable for D_2O), 6.15−5.8 (m, 2H, 2 × CH_2-C*H*═), 5.25−4.9 (m, 4H, 2 × CH═CH_2), 4.6 (d, 2H, N-CH_2-CH═), 4.4 (s, 1H, N-C*H*), 4.25−4.0 (m, 4H, CH_2-N-CH_2), 3.8 (t, 2H, NH-CH_2-CH═, d after exchange for D_2O), 2.35 (m, 4H, CH_2-N-CH_2). Anal. ($C_{28}H_{29}F_2N_7$) C, H, N.

9-Allyl-6-(allylamino)-2-[4-[bis(4-fluorophenyl)methyl]-piperazin-1-yl]purine (37). A representative procedure is as follows for the conversion mentioned with the eluting system in parentheses for flash chromatography: **74** → **38** (76%) (CH_2Cl_2−MeOH, 95:5). A solution of **40** (1.7 g, 0.0068 mol), 1-[bis(4-fluorophenyl)methyl]piperazine (3.9 g, 0.0136 mol), potassium iodide (0.05 g), and ethyl alcohol (50 mL) was heated for 24 h at 150 °C in an autoclave. The solvent was evaporated in vacuo, and the residue was treated with CH_2Cl_2 and a 10% aqueous Na_2CO_3 solution. The organic layer was separated, washed with water, dried ($MgSO_4$), and concentrated. The residue was crystallized from isopropyl ether to provide 1.2 g (35%) of the title compound **37**: mp 208−209 °C; IR (Nujol) 3276−3200 (NH), 1601 cm^{-1} (C═N); ^1H NMR (DMSO-d_6, 200 MHz) δ7.7 (s, 1H, N═C*H*-N), 7.45 (m, 4H, aromatic *H*), 7.15 (m, 4H, aromatic *H*), 6.15−5.8 (m, 2H, 2 × C*H*═CH_2), 5.2−5.0 (m, 4H, 2 × CH═CH_2), 4.6 (d, 2H, N-CH_2-CH═), 4.35 (s, 1H, N-C*H*), 4.05 (m, 2H, HN-CH_2-CH═), 3.7 (m, 4H, CH_2-N-CH_2), 2.3 (m, 4H, CH_2-N-CH_2). Anal. ($C_{28}H_{29}F_2N_7$) C, H, N.

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