Synthesis of a Series of 3-Cyanopropionamides and 4-Imino-γ-butyrolactams and Evaluation of their Function as Modulators of Multidrug Resistance [1)]

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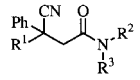
Key Words: *Synthesis of 2,2,2-trialkyl-3-cyano amides; synthesis of 4-imino-γ-butyrolactams (5-iminopyrrolidin-2-ones); highly branched multidrug-resistance (MDR) modulators*

Summary

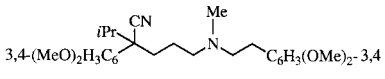
The synthesis of the 3-cyanopropionamides **3a** and **3b**, of the 2,2-dimethyl-3-cyanopropionamides **4a**–**4c** and of the 4-imino-γ-butyrolactams **5a** and **5b** (cyclic functional isomers of 3-cyanopropionamides) is described. The amides **3a** and **3b** were obtained by aminolysis of the corresponding acid chlorides, which are accessible via hydrolysis of the ethyl esters to the acids. This methodology was not used for the synthesis of the amides **4a**–**4c** owing to steric hindrance to hydrolysis in the corresponding ethyl esters. These nonreactive esters, accesible by alkylation of 1-cyano carbanions with ethyl bromodimethylacetate, could be directly converted into the amides **4a**–**4c** by aminolysis with the lithium amide of 3,4-dimethoxy-*N*-methylphenethylamine. Instead of open-chain amides, the lactams **5a** and **5b** are obtained when the lithium amide of 3,4-dimethoxyphenethylamine (i.e., of a primary rather than secondary amine) is used for the aminolysis. The synthesized compounds were tested for their ability to decrease the resistance to vincristine in a multidrug-resistant subline of murine leukemic lymphoblasts that are 300-fold resistant to the antiproliferative drug. The amides **4a** and **4c**, and lactam **5a**, all of which have a highly branched carbon backbone, were active. Lactam **5a** reduced the vincristine resistance by 90% at a 2-μM concentration.

Introduction

Multidrug resistance (MDR) is a major obstacle for an effective tumor chemotherapy [1,2]. This type of cross-resistance to a variety of antitumor drugs can be induced by a single antitumor drug during patient treatment. We have reported the synthesis of the 3-cyanopropionamides **1a**–**1e** together with an evaluation of their function as modulators of MDR [3,4]. These amides possess certain structural features of the coronary vasodilator verapamil (**2**) whose ability to modulate MDR is well-known [5]. Thus, both the amides and verapamil bear a cyano group on a quaternary carbon atom, and a phenethyl group on a nitrogen atom. We found that amide **1b** diminished the resistance of an MDR murine cell subline to the antitumor drug vincristine to an extent similar to that effected by verapamil [3]. In order to enlarge the scope of that work, we now synthesized the new 3-cyanopropionamides **3a**, **3b**, and **4a**–**4c** and evaluated their MDR-modulating function.



	R^1	R^2	R^3
1a	Ph	H	3,4-$(MeO)_2C_6H_3CH_2CH_2$
1b	4-$O_2NC_6H_4$	Me	3,4-$(MeO)_2C_6H_3CH_2CH_2$
1c	4-$O_2NC_6H_4$	H	PhCH(OH)CHMe
1d	3,4-$(MeO)_2C_6H_3$	Me	3,4-$(MeO)_2C_6H_3CH_2CH_2$
1e	3,4-$(MeO)_2C_6H_3$	H	PhCH(OH)CHMe



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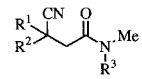
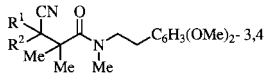


	R^1	R^2	R^3
3a	Ph	4-$O_2NC_6H_4$	$PhCH_2CH_2$
3b	*i*Pr	3,4-$(MeO)_2C_6H_3$	3,4-$(MeO)_2C_6H_3CH_2CH_2$
3c	Ph	2-Pyridyl	3,4-$(MeO)_2C_6H_3CH_2CH_2$



	R^1	R^2
4a	Ph	Ph
4b	Ph	3,4-$(MeO)_2C_6H_3$
4c	*i*Pr	3,4-$(MeO)_2C_6H_3$
4d	Ph	4-$O_2NC_6H_4$

The new amides share with the previously prepared amides and with verapamil the above-mentioned structural features. However, we now varied the *N*-(3,4-dimethoxyphenethyl) group in active **1b** by the unsubstituted *N*-phenethyl group allowing structure **3a**, to evaluate the role of the former group for activity. Amide **3b** is the closest analogue of verapamil in the whole series. The amides **4a**–**4c** feature an additional quaternary carbon atom, which bears two newly introduced methyl groups and is adjacent to the pre-existing quaternary carbon. These amides are branched-chain counterparts of the

[1)] Branched-Chain Carbon Compounds, 6. Part 5: Ref.[10]

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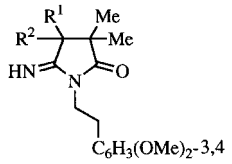
amides **1a**, **1d**, and **3a**, and are more lipophilic than the latter ones. In addition, steric constraints arising from the densely branched carbon backbone may restrict the conformational flexibility of **4a**–**4c** [6]. We herein present NMR support for such a view. An increased lipophilicity and a restricted conformational flexibility may facilitate an interference of **4a**–**4c** with MDR-mediator P-glycoprotein. This plasma-membrane glycoprotein, which is overexpressed in MDR cells, mediates the expulsion of lipophilic antitumor drugs out of the cells [1,2]. This detrimental function is modulated by verapamil by means of its binding to the glycoprotein [7]. Verapamil is a flexible molecule [8] and studies on less flexible verapamil analogues seeking an improved therapeutic efficacy have been carried out [9]. The synthesis of **3c** and **4d**, which are a simple heterocyclic analogue of active **1b** and a branched-chain counterpart of **1b**, respectively, was unsuccessful.



	R^1	R^2
5a	Ph	3,4-$(MeO)_2C_6H_3$
5b	*i*Pr	3,4-$(MeO)_2C_6H_3$

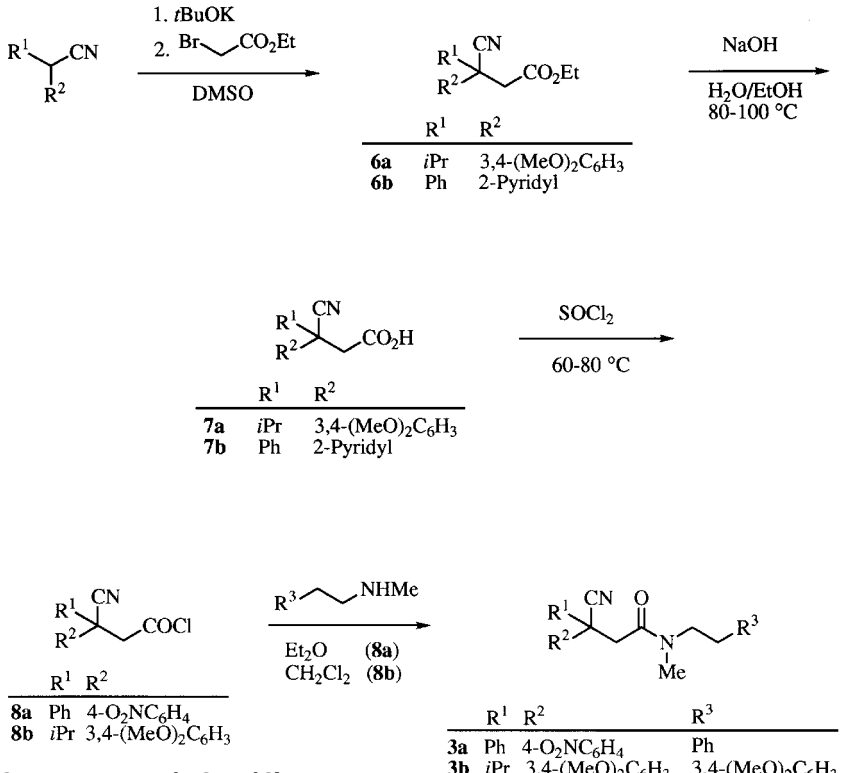
In the course of the synthetic work on branched-chain amides, we obtained the heavily substituted 4-imino-γ-butyrolactams **5a** and **5b** in an unplanned way. These lactams may be regarded as cyclic tautomers of 3-cyanopropionamides having an *N*-hydrogen. We have recently reported the synthesis of **5b** [10]. The lactams **5a** and **5b** were biologically tested along with the amides **3a**, **3b**, and **4a**–**4c**.

Results and Discussion

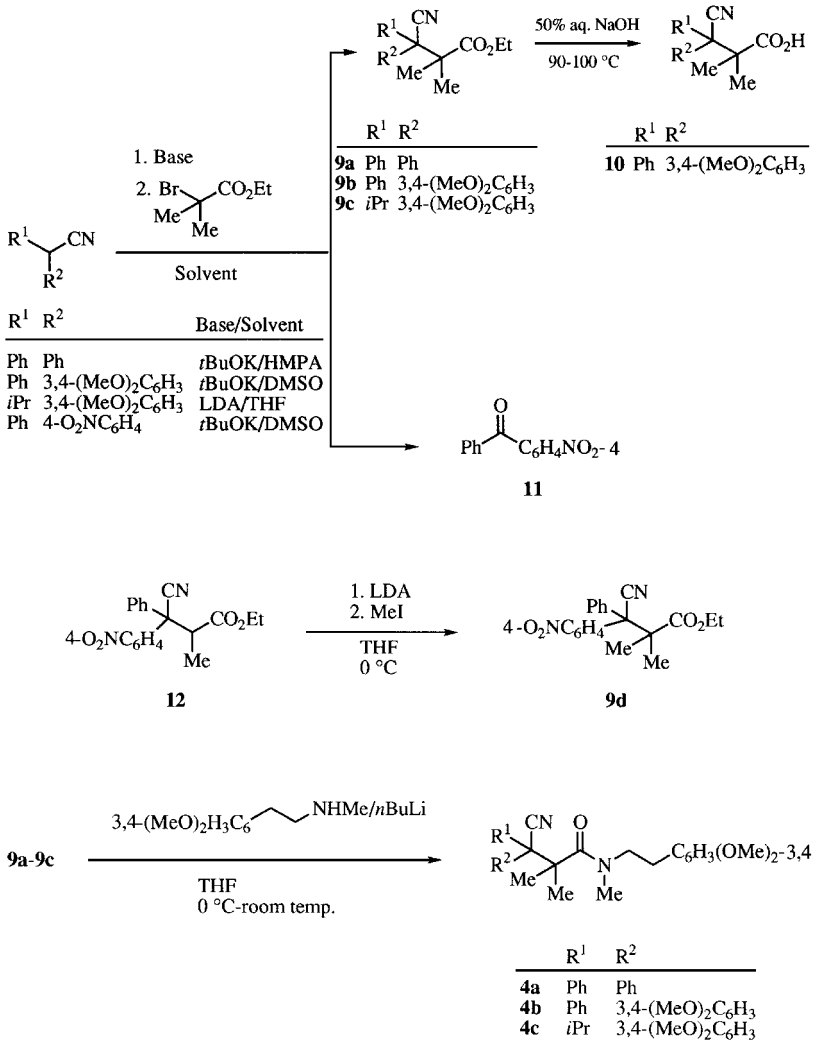
Synthesis

The 3-cyanopropionamides **3a** and **3b** were obtained by aminolysis of the acid chlorides **8a** and **8b** (Scheme 1). We previously used this procedure for the amides **1a**–**1e** [3,4]. At that time, we prepared **8a** in a stepwise manner, and we now prepared **8b** by the same methodology (Scheme 1). Compared with the previous alkylations of 1-cyano carbanions with ethyl bromoacetate [3,4], that for the carbanion of isopropyl(3,4-dimethoxyphenyl)acetonitrile[11] gave a low yield of the alkylation product (**6a**), probably as a result of a nonquantitative generation of the strongly basic carbanion by *tert*-butoxide ion, since a large amount of unreacted nitrile was recovered. Alkaline hydrolysis of **6a** to acid **7a** and subsequent treatment of the acid with thionyl chloride proceeded without difficulties. Aiming at obtaining 3-(2-pyridyl)-propionamide **3c**, we prepared ester **6b** and acid **7b**. However, treatment of **7b** with thionyl chloride gave a crude black solid from which we did not succeed in isolating the corresponding acid chloride.

With the idea of synthesizing the 2,2-dimethyl-3-cyanopropionamides **4a**–**4d** by the usual methodology, we first undertook the preparation of the pertinent 2,2-dimethyl-3-cyanopropionates (**9**) by alkylation of 1-cyano carbanions with ethyl bromodimethylacetate (Scheme 2). The alkylation of 1-cyano carbanions with 2-bromo-2,2-dialkyl esters (a sort of sterically retarded nucleophilic substitutions at a tertiary



Scheme 1. Synthesis of the 3-cyanopropionamides **3a** and **3b**.

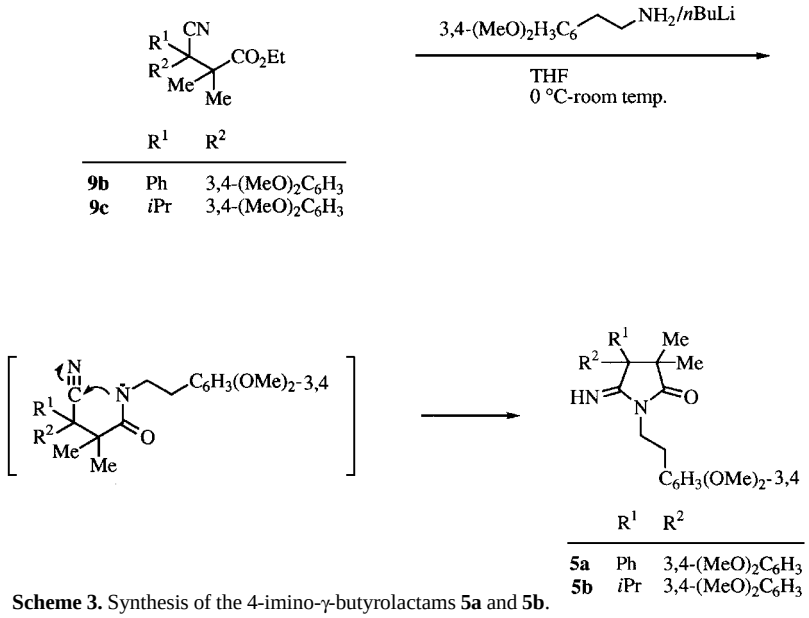


Scheme 2. Synthesis of the 2,2-dimethyl-3-cyanopropionamides **4a–4c**.

carbon atom) is feasible on literature grounds [12]. It has been reported that alkylation of diphenylacetonitrile by ethyl bromodimethylacetate using sodium amide as base in benzene gives a moderate yield of **9a** (50–50%) [13]. We could significantly increase the yield of this reaction (87%) using instead potassium *tert*-butoxide as base in HMPA. We found potassium *tert*-butoxide to be satisfactory for **9b** as well. For **9c**, we used the stronger base lithium diisopropylamide in order to ensure a quantitative generation of the above-mentioned carbanion of isopropyl-(3,4-dimethoxyphenyl)acetonitrile; nevertheless, the yield was low [6]. The strongly stabilized and weakly nucleophilic carbanion of phenyl-(4-nitrophenyl)acetonitrile failed to be alkylated by ethyl bromodimethylacetate; instead of the alkylation product, we obtained 4-nitrobenzophenone (**11**) from a sluggish oxidation of the carbanion in the reaction medium. In an alternative manner, we could obtain a small sample of the desired 3-(4-nitrophenyl) ester (**9d**) by α-alkylation of 3-(4-nitrophenyl) ester **12** by methyl iodide.

The saponification of 2,2,2-trialkyl ester **9a** has been reported [13]. However, we found it very difficult to saponify the closely analogous ester **9b**. Under the conditions used to saponify the 2,2-unsubstituted esters **6a** and **6b**, ester **9b** remained unaffected. We ascribe this resistance to hydrolysis to a high steric hindrance arising from the very bulky tertiary alkyl group adjacent to the carbonyl group. Prolonged heating of the ester in 50% aqueous sodium hydroxide gave only a low yield of the acid (**10**) and the glass reaction flask was seriously attacked under these conditions. In the light of these difficulties, we stopped trying the synthesis of the amides **4a**–**4d** by the usual methodology.

Following a literature procedure for aminolysis of nonreactive 2,2,2-trialkyl esters employing lithium amides [14], we obtained the 2,2,2-trialkyl amides **4a**–**4c** from the esters **9a**–**9c** with the lithium amide generated from 3,4-dimethoxy-*N*-methylphenethylamine (Scheme 2). The yields were low (29–42%). In striking constrast to the esters **9a**–**9c**, 3-(4-nitrophenyl) ester **9d** was totally unaffected by the lithium amide, an event that prevented the preparation of branched-chain 3-(4-nitrophenyl) amide **4d**. We interpret this surprising result by the formation of a charge-transfer complex between the reactants, involving the electron-deficient nitro aromatic ring of the ester as electron acceptor and the anion as electron donor. Actually, a reddish-brown coloration developed on mixing the ester with the lithium amide. The stability of such proposed complex in the medium (Li^+/THF)



	R^1	R^2
9b	Ph	3,4-$(MeO)_2C_6H_3$
9c	*i*Pr	3,4-$(MeO)_2C_6H_3$

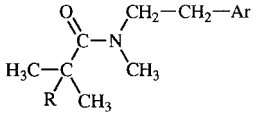
	R^1	R^2
5a	Ph	3,4-$(MeO)_2C_6H_3$
5b	*i*Pr	3,4-$(MeO)_2C_6H_3$

Scheme 3. Synthesis of the 4-imino-γ-butyrolactams **5a** and **5b**.

should be high enough to preclude the aminolysis reaction. Charge-transfer complexes of aromatic nitro compounds with bases are known [15].

When we carried out the reaction of the esters **9b** and **9c** with the lithium amide of 3,4-dimethoxyphenethylamine (i.e., of a primary rather than secondary amine), we obtained the 4-imino-γ-butyrolactams **5a** and **5b** (Scheme 3), in place of the desired branched-chain 3-cyanopropionamides bearing only a single *N*-alkyl group. The *N*-alkyl amides probably are reaction intermediates, which would isomerize to the lactams by ring closure of the amide ions resulting from loss of the acidic *N*-proton under the basic reaction conditions. Such an isomerization process is not possible for the *N*,*N*-dialkyl amides **4a**–**4c** lacking an *N*-proton. Again, 3-(4-nitrophenyl) ester **9d** failed to react with a lithium amide.

Both *e* and *z* amide rotamers of the amides **3a** and **3b** exist in solution in significant amounts, as shown by the ^{1}H NMR spectra. For *N*,*N*-dialkyl amides, such as **3a** and **3b**, it may be expected on steric grounds that both rotamers will exist, while *N*-alkyl amides may have a strong steric preference for the *z* rotamer.[3,4] It was thus interesting that the ^{1}H NMR spectra of the *N*,*N*-dialkyl amides **4a**–**4c** showed only a single rotamer. We ascribe this result to the exclusive existence (within experimental NMR sensitivity) of the less sterically demanding *z* rotamer [see (*z*)-**4a**–**4c**], rather than to a fast interconversion between the rotamers giving rise to averaged NMR spectra. In the amides **4a**–**4c**, the very bulky tertiary alkyl group attached to the amide carbonyl carbon sterically prevents the *e* rotamer from existing. Hence these branched-chain amides have a restricted conformational flexibility, regarding this property as a diminished number of existing



(*z*)-**4a**-**4c**

conformers. Binding of these amides to P-glycoprotein may thus be facilitated if they contain the suitable conformers.

Biological Test

The compounds synthesized were tested for the ability to decrease the resistance of MDR P400 cell subline [16,17] to vincristine. This MDR subline of the L5178Y murine leukemic lymphoblasts is very resistant to vincristine: the cell-growth inhibition by the antiproliferative drug on the resistant and the parental cells is (expressed as the IC_{50} value) 2.5 μM and 8.3×10^{-3} μM, respectively, thus the relative resistance of the resistant cells to the drug amounts to 300. We measured the enhancement of vincristine antiproliferative activity on the resistant cells, as brought about by the compounds, by determining the vincristine IC_{50}'s in the presence of the compounds and comparing the values with that in the absence of compounds. The effect of the compounds on vincristine activity was also measured in the parental cells.

Prior to the test, the cell-growth-inhibitory effect of the compounds was established in order to select concentrations of the compounds that would not interfere with cell growth in the test. Employing such concentrations, the effect on vincristine activity, measured as above described, neatly reflects the enhancing activity of the compounds. The IC_{50}'s of the compounds were determined to be > 30 μM in all the cases (Table 1). Considering this IC_{50} threshold, we tested the compounds at lower 0.7-, 2-, and 7-μM concentrations in a uniform manner; above 7 μM, the cell-growth inhibitions are important. It is worth noting that the compounds inhibit cell growth of the MDR cells to a lesser extent than the MDR-modulator verapamil in the same fashion as the previous compounds **1a**–**1e** [3,4], which is an interesting characteristic of the whole series of compounds. In particular, the inhibition by the closest verapamil analogue **3b** is 40-fold weaker than that by verapamil.

Table 1. Inhibition of cell growth by compounds (IC_{50}) on MDR P400 cells and on parental L5178Y cells.

Compound	IC_{50}[a] [μM] MDR P400 cells	Parental L5178Y cells
3a	> 30[b]	> 30[b]
3b	840	730
4a	> 30[b]	> 30[b]
4b	65	71
4c	120	160
5a	> 30[b]	> 30[b]
5b	190	200
verapamil (**2**)	19[c]	> 7[c]

[a] Values shown are the average of at least two experiments performed with replicate concentrations; relative standard deviation < |40%|.– [b] At 30 μM, inhibition of cell growth was < 30%; the compound was insoluble at > 30 μM.– [c] Ref.[3].

The vincristine IC_{50}'s in the presence of the compounds for the MDR and the parental cells are gathered in Table 2; the values for the compounds **1a**–**1e**, previously determined in the same systems [3,4], have been included in the table to survey the whole series of compounds. We express the enhancing effect of the compounds on vincristine activity as the ratio of the vincristine IC_{50} value in the absence of compound to the value in the presence of compound; the ratios are shown in parentheses in the table. We classify a compound as inactive if it manifested no enhancing effect (corresponding to a value of 1) at the lowest test concentration, even if the compound was effective at a higher test concentration.

Among the compounds that were tested now, the branched-chain amides **4a** and **4c**, and lactam **5a** are active in the MDR cells according to our criterion. The activity of **4a** and **4c** is similar to that of the previously tested **1b** and verapamil, while that of **5a** is higher. The structural variation of **3a** resulted in loss of the activity: all the active compounds, including verapamil, bear an *N*-(3,4-dimethoxyphenethyl) group, which thus seems to be a structural factor for the activity in this series of compounds. On the other hand, the closest verapamil analogue **3b** was ineffective, even at the highest test concentration. This inactivity of **3b** parallels its low cell-growth-inhibitory effect as above mentioned. An activity arose, however, on branching out the carbon backbone in structure **3b** allowing structure **4c**. Considering the whole series, activity is more frequently a property of the compounds having a highly branched carbon backbone, such as **4a**, **4c**, and **5a**, which seems to indicate that this structural feature is beneficial for the activity. As indicated early in this paper, MDR modulation may be favored by an increased lipophilicity and restricted conformational flexibility as conferred on a modulator by a highly branched carbon backbone.

A property that distinguishes 4-imino-γ-butyrolactam **5a**, the most active tested compound, from the amides is its higher basicity resulting from the amidino group (N-C=NH) in the structure. The lactam is soluble in aqueous hydrogen chloride at pH < 5, in contrast to the amides. It has been reported that the presence of a basic nitrogen atom is an important structural feature for MDR modulators [8], which is consistent with our results.

In the parental cells, some of the tested compounds were active. However, the activity of these compounds was lower than that for lactam **5a** in the MDR cells. Furthermore, the compounds active in the MDR cells were consistently inactive or less active in the parental cells, in agreement with a common behavior of MDR modulators [18,19].

As a final conclusion of this work, we present in Table 3 the values of relative resistance of MDR P400 cell subline to vincristine in the presence of the active compounds. A full circumvention of the high vincristine resistance of this subline (an event corresponding to a relative resistance of 1) was not observed with these compounds. Nevertheless, the most active compound, lactam **5a**, achieved a 67 to 97% reduction of the resistance at moderate 0.7- to 7-μM pharmacological concentrations.

Table 2. Vincristine IC_{50}'s in the presence of compounds in MDR P400 cells and in parental L5178Y cells.

Compound	Vincristine IC_{50}[a] [μM] MDR P400 cells Concentration of compound [μM]: 0	0.7	2	7	[10^{-3} μM] Parental L5178Y cells 0	0.7	2	7
1a	3.0 (1)	3.3[b] (1)	1.5[b] (2)		5.7 (1)	3.0[b] (2)	1.9[b] (3)	
1b	2.5 (1)	1.2[b] (2)	0.71[b] (3)		6.3 (1)	7.5[b] (1)	4.5[b] (1)	
1c	2.8 (1)	2.9[c] (1)	1.4[c] (2)		5.4 (1)	3.1[c] (2)	2.3[c] (2)	
1d	2.3 (1)	1.8[c] (1)	1.2[c] (2)		4.5 (1)	3.8[c] (1)	1.9[c] (2)	
1e	2.7 (1)	2.1[c] (1)	1.5[c] (2)		6.4 (1)	6.1[c] (1)	4.6[c] (1)	
3a	3.0 (1)	2.4 (1)	1.5 (2)	0.77 (4)	8.2 (1)	9.7 (1)	9.0 (1)	4.3 (2)
3b	1.2 (1)	1.1 (1)	0.92 (1)	0.82 (1)	8.4 (1)	6.6 (1)	7.8 (1)	6.4 (1)
4a	2.0 (1)	0.83 (2)	0.38 (5)	0.21 (9)	13 (1)	8.5 (1)	7.8 (2)	5.2 (2)
4b	2.4 (1)	2.1 (1)	1.3 (2)	0.19 (10)	16 (1)	8.0 (2)	7.2 (2)	3.5 (4)
4c	3.6 (1)	1.9 (2)	0.72 (5)	0.21 (20)	10 (1)	5.3 (2)	3.0 (3)	1.6 (6)
5a	2.9 (1)	1.0 (3)	0.26 (10)	0.10 (30)	9.5 (1)	4.4 (2)	4.1 (2)	2.8 (3)
5b	1.7 (1)	1.5 (1)	1.1 (1)	0.29 (6)	8.7 (1)	7.2 (1)	3.9 (2)	2.0 (4)
Verapamil (**2**)	2.8 (1)	1.4[b] (2)	0.99[b] (3)		6.2 (1)	8.2[b] (1)	2.8[b] (2)	

[a] For the compounds **3a**, **3b**, **4a**–**4c**, **5a**, and **5b**, the values are the average of at least two experiments performed with replicate concentrations; relative standard deviation < |40%|; the accompanying number in parentheses is the ratio of the corresponding control IC_{50} in the absence of the compound to the IC_{50} in the presence of the compound.– [b] Ref.[3].– [c] Ref.[4].

Table 3. Relative resistance of MDR P400 cells to vincristine in the presence of compounds modulating the resistance.

Compound	Relative resistance[a] Concentration of compound [μM]: 0	0.7	2	7
none	300			
1b		100	80	
4a		100	60	30
4c		200	60	20
5a		100	30	10
Verapamil (**2**)		100	100	

[a] Relative resistance is expressed as the ratio of vincristine IC_{50} value in the presence of compound in MDR P400 cells to the average value in the absence of compounds in parental L5178Y cells (8.3×10^{-3} μM); the former values as given in Table 2 have been corrected to anchor them all to the same average control IC_{50} (2.5 μM).

Acknowledgments

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Experimental

General

Isopropyl-(3,4-dimethoxyphenyl)acetonitrile,[11] phenyl-(2-pyridyl)acetonitrile,[20] **8a**,[3] *N*-methylphenethylamine,[21] phenyl-(3,4-dimethoxyphenyl)acetonitrile,[22] phenyl-(4-nitrophenyl)acetonitrile[23] and **9c**[6] were prepared according to literature procedures. Dry Et_2O was prepared with sodium wire. HMPA and THF were dried by distillation from CaH_2 and $LiAlH_4$, respectively, and were stored over 3-A molecular sieves; dry DMSO (distilled from CaH_2) was occasionally used. A commercial solution of *n*BuLi in *n*-C_6H_{14} was titrated with MeOH at 0 °C under nitrogen, using dry THF as co-solvent and 2,2′-dipyridyl as indicator; it was found to be 2.0 M. For the reactions involving the use of *t*BuOK or *n*BuLi, transfers were effected by means of syringe.– Column chromatography: MN Silica Gel 60.– TLC: Merck Silica Gel 60 F_{254}.– Mp: Reichert-Jung Thermovar.– IR: Perkin-Elmer 681.– NMR: Perkin-Elmer EM 390, Varian Gemini, Bruker AM 200, Varian Inova, Varian Unity 500. For ^{1}H NMR, TMS as internal standard; for ^{13}C NMR, $CDCl_3$ δ_C = 77.0.

*Ethyl 3-Cyano-3-isopropyl-3-(3,4-dimethoxyphenyl)propionate (**6a**)*

To a solution of 3.85 g (34.3 mmol) of *t*BuOK in 20 ml of DMSO, a solution of 7.00 g (32.0 mmol) of isopropyl-(3,4-dimethoxyphenyl)acetonitrile in 40 ml of DMSO was added dropwise with stirring at room temp. under nitrogen, followed by addition of 3.5 ml (31 mmol) ethyl bromoacetate. After 0.5 h, ice was added, the mixture was extracted with Et_2O, and the ethereal phase was washed with water and dried (Na_2SO_4). Column chromatography on silica gel with petroleum ether/Et_2O (3:2) as eluent (R_f = 0.2) gave 1.98 g of **6a** (21%), thick liquid.– ^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.86 [d, *J* = 7 Hz, 3 H, $CH(CH_3)_2$], 1.07 (t, *J* = 7 Hz, 3 H, OCH_2CH_3), 1.20 [d, *J* = 7 Hz, 3 H, $CH(CH_3)_2$], 2.16 [sept, *J* = 7 Hz, 1 H, $CH(CH_3)_2$], 2.87 (d, *J* = 16 Hz, 1 H, 2-H), 3.18 (d, *J* = 16 Hz, 1 H, 2-H), 3.89 (s, 3 H, OCH_3), 3.91 (s, 3 H, OCH_3), 3.98 (q, *J* = 7 Hz, 2 H, OCH_2CH_3), 6.9 (m, 3 H, aromatic H).– Anal. ($C_{17}H_{23}NO_4$) C, H, N.

*Ethyl 3-Cyano-3-phenyl-3-(2-pyridyl)propionate (**6b**)*

The procedure for **6a** was followed, using 6.06 g (54.0 mmol) of *t*BuOK in 50 ml of DMSO, 10.0 g (51.5 mmol) of phenyl-(2-pyridyl)acetonitrile in 100 ml of DMSO, and 7.9 ml (71 mmol) of ethyl bromoacetate. Ice was added after the reaction, and the solid precipitate formed was filtered out and washed with water. Recrystallization from EtOH gave 10.2 g of **6b** (71%), mp 87–88 °C.– ^{1}H NMR (90 MHz, $CDCl_3$): δ = 1.08 (t, *J* = 7 Hz, 3 H, OCH_2CH_3), 3.31 (d, *J* = 17 Hz, 1 H, 2-H), 3.85 (d, *J* = 17 Hz, 1 H, 2-H), 4.02 (q, *J* = 7 Hz, 2 H, OCH_2CH_3), 7.2–7.8 (m, 8 H, aromatic H), 8.5 (m, 1 H, aromatic H).– Anal. ($C_{17}H_{16}N_2O_2$) C, H, N.

*3-Cyano-3-isopropyl-3-(3,4-dimethoxyphenyl)propionic Acid (**7a**)*

A stirred mixture of 1.04 g (3.41 mmol) of **6a**, 0.50 g (12 mmol) of NaOH, and 15 ml of water/EtOH (3:2) was heated at 80–100 °C for 1 h. The mixture was then diluted with water, washed with Et_2O to remove unreacted **7a**, and acidified (pH = 1) with concd. aqueous HCl. The formed suspension separated out in the form of a crystalline precipitate upon addition of NaCl, and the crystals were filtered out and washed with water to give 0.860 g of **7a** (91%), mp 103–104 °C.– ^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.81 [d, *J* = 7 Hz, 3 H, $CH(CH_3)_2$], 1.15 [d, *J* = 7 Hz, 3 H, $CH(CH_3)_2$], 2.12 [sept, *J* = 7 Hz, 1 H, $CH(CH_3)_2$], 2.88 (d, *J* = 16 Hz, 1 H, 2-H), 3.16 (d, *J* = 16 Hz, 1 H, 2-H), 3.85 (s, 3 H, OCH_3), 3.87 (s, 3 H, OCH_3), 6.8–7.0 (m, 3 H, aromatic H).– Anal. ($C_{15}H_{19}NO_4$) C, H, N.

*3-Cyano-3-phenyl-3-(2-pyridyl)propionic Acid (**7b**)*

A stirred mixture of 9.61 g (34.3 mmol) of **6b**, 4.80 g (120 mmol) of NaOH and 140 ml of water/EtOH (3:2) was heated at 100 °C for 0.5 h. The mixture was then diluted with water and brought to pH = 7 with concd. aqueous HCl. The precipitate formed was filtered out and washed with water to give 7.19 g of **7b** (83%), mp 95–105 °C (dec.) .– ^{1}H NMR (200 MHz, $CDCl_3$): δ = 3.38 (d, *J* = 17 Hz, 1 H, 2-H), 3.88 (d, *J* = 17 Hz, 1 H, 2-H), 7.2–7.8 (m, 8 H, aromatic H), 8.6 (m, 1 H, aromatic H), 10.4 (br., 1 H, CO_2H).– Anal. ($C_{15}H_{12}N_2O_2$) C, H, N.

*3-Cyano-3-isopropyl-3-(3,4-dimethoxyphenyl)propionyl Chloride (**8b**)*

A mixture of 400 mg (1.44 mmol) of **7a** and 1.0 ml (13 mmol) of redistilled $SOCl_2$ was heated at 60–80 °C for 1 h. Then, unreacted $SOCl_2$ was evaporated. Recrystallization of the residue from petroleum ether/CH_2Cl_2 gave 319 mg of **8b** (75%), mp 115–117 °C.– ^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.83 [d, *J* = 7 Hz, 3 H, $CH(CH_3)_2$], 1.19 [d, *J* = 7 Hz, 3 H, $CH(CH_3)_2$], 2.16 [sept, *J* = 7 Hz, 1 H, $CH(CH_3)_2$], 3.51 (d, *J* = 18 Hz, 1 H, 2-H), 3.73 (d, *J* = 18 Hz, 1 H, 2-H), 3.88 (s, 3 H, OCH_3), 3.91 (s, 3 H, OCH_3), 6.8–7.0 (m, 3 H, aromatic H).– Anal. ($C_{15}H_{18}ClNO_3$) C, H, N.

*3-Cyano-3-phenyl-3-(4-nitrophenyl)-N-methyl-N-phenethylpropionamide (**3a**)*

To a solution of 157 mg (1.16 mmol) of *N*-methylphenethylamine in 1 ml of dry Et_2O, a solution of 183 mg (0.582 mmol) of **8a** in 5 ml of dry Et_2O was slowly added with stirring at room temp. After 1.5 h, water was added to digest the precipitate formed. The undissolved solid was filtered off, and washed with water and then with a little Et_2O. Recrystallization from Et_2O/CH_2Cl_2 gave 87 mg of **3a** (36%), mp 127–129 °C.– IR (KBr): ν = 1650 cm^{-1} (s, CO).– ^{1}H NMR (200 MHz, $CDCl_3$; rotameric ratio = 3:2): δ = 2.79 and 2.90 (2 t, *J* = 7 Hz, 2 H, NCH_2CH_2Ph); 2.84 and 2.95, and 3.40 [2 d (*J* = 18 Hz) and 1 s, 2 H, 2-H]; 2.90 and 2.97 (2 s, 3 H, NCH_3); 3.4–3.7 (m, 2 H, NCH_2CH_2Ph); 7.0–7.5 (m, 10 H, Ph); 7.17 and 7.55 (2 d, *J* = 9 Hz, 2 H, aromatic H); 8.11 and 8.21 (2 d, *J* = 9 Hz, 2 H, aromatic H).– Anal. ($C_{25}H_{23}N_3O_3$) C, H, N.

*3-Cyano-3-isopropyl-3-(3,4-dimethoxyphenyl)-N-methyl-N-(3,4-dimethoxyphenethyl)propionamide (**3b**)*

To a solution of 348 mg (1.78 mmol) of 3,4-dimethoxy-*N*-methylphenethylamine in 3 ml of CH_2Cl_2, a solution of 254 mg (0.860 mmol) of **8b** in 1 ml of CH_2Cl_2 was slowly added. After 0.5 h, the resulting solution was washed with water and dried (Na_2SO_4). Separation by column chromatography on silica gel with C_6H_6/AcOEt (1:1) as eluent (R_f = 0.4) gave 290 mg of **3b** (74%), noncrystalline solid.– IR (KBr): ν = 2240 cm^{-1} (w, C≡N), 1650 (s, C=O).– ^{1}H NMR (200 MHz, $CDCl_3$; rotameric ratio = 3:2): δ = 0.72 and 0.82 [2 d, *J* = 7 Hz, 3 H, $CH(CH_3)_2$]; 1.03 and 1.20 [2 d, *J* = 7 Hz, 3 H, $CH(CH_3)_2$]; 2.18 and 2.37 [2 sept, *J* = 7 Hz, 1 H, $CH(CH_3)_2$]; 2.60 and 2.67 (2 t, *J* = 8 Hz, 2 H, NCH_2CH_2Ar); 2.60, and 2.91 and 3.02 [1 s and 2 d (*J* = 15 Hz), 2 H, 2-H); 2.70 and 2.80 (2 s, 3 H, NCH_3); 3.3–3.5 (m, 2 H, NCH_2CH_2Ar); 3.81, 3.82, 3.84 and 3.86 (4 s, 12 H, OCH_3); 6.5–7.0 (m, 6 H,

aromatic H). ^{13}C NMR (50 MHz, $CDCl_3$): δ = 19.0, 19.1, 19.3, 19.4, 33.8, 34.2, 34.9, 37.0, 37.2, 37.4, 40.2, 40.8, 50.8, 51.0, 51.3, 52.4, 56.5, 56.6, 56.7, 110.9, 111.1, 111.6, 111.7, 112.0, 112.5, 112.7, 118.6, 119.2, 121.4, 121.5, 121.8, 131.2, 131.3, 132.2, 149.0, 149.2, 149.4, 149.5, 149.7, 150.0, 168.4, 168.6.– Anal. ($C_{26}H_{34}N_2O_5$) C, H, N.

*Ethyl 2,2-Dimethyl-3-cyano-3,3-diphenylpropionate (**9a**)*

The preparation of **9a** by a procedure anologous to the following one has been reported in a short communication providing no characterization data.[13]

To a solution of 0.25 g (2.2 mmol) of *t*BuOK in 6 ml of dry HMPA, a solution of 410 mg (2.12 mmol) of diphenylacetonitrile in 2 ml of dry HMPA was added dropwise with stirring at room temp. under nitrogen. The resulting solution was cooled to 0 °C, and 0.32 ml (2.1 mmol) of ethyl bromodimethylacetate was added. After 2.5 h, the mixture was diluted with water and extracted with Et_2O, and the ethereal phase was thoroughly washed with water to remove the HMPA, and dried (Na_2SO_4). Kugelrohr distillation (oven temp. 239–240 °C/0.5 Torr) gave 563 mg of **9a** (87%), thick liquid that crystallized on prolonged standing, mp 51–53 °C.– ^{1}H NMR (90 MHz, $CDCl_3$): δ = 0.98 (t, *J* = 7 Hz, 3 H, OCH_2CH_3), 1.37 (s, 6 H, 2-CH_3), 4.03 (q, *J* = 7 Hz, 2 H, OCH_2CH_3), 7.1–7.4 (m, 10 H, aromatic H).– Anal. ($C_{20}H_{21}NO_2$) C, H, N.

*Ethyl 2,2-Dimethyl-3-cyano-3-phenyl-3-(3,4-dimethoxyphenyl)propionate (**9b**)*

The procedure for **6a** was followed using 0.51 g (4.5 mmol) of *t*BuOK in 6 ml of DMSO, 1.14 g (4.52 mmol) of phenyl-(3,4-dimethoxyphenyl)acetonitrile in 6 ml of DMSO, 0.67 ml (4.5 mmol) of ethyl bromodimethylacetate and a 5-h reaction time. Column chromatography on silica gel with petroleum ether/EtO_2 (7:3) as eluent (R_f = 0.2) gave 1.15 g of **9b** (69%),viscous liquid that crystallized on prolonged standing, mp 66–70 °C.– ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.05 (t, *J* = 7 Hz, 3 H, OCH_2CH_3), 1.40 (s, 3 H, 2-CH_3), 1.45 (s, 3 H, 2-CH_3), 3.78 (s, 3 H, OCH_3), 3.88 (s, 3 H, OCH_3), 4.09 (q, *J* = 7 Hz, 2 H, OCH_2CH_3), 6.7–7.0 (m, 3 H, aromatic H), 7.2–7.5 (m, 5 H, aromatic H).– Anal. ($C_{22}H_{25}NO_4$) C, H, N.

*2,2-Dimethyl-3-Cyano-3-phenyl-3-(3,4-dimethoxyphenyl)propionic Acid (**10**)*

A stirred mixture of 99 mg (0.27 mmol) of **9b** and 4 ml of 50% aqueous NaOH was heated at 90–100 °C for one week. An abundant solid was formed by attack of the alkali on the glass flask. The mixture was diluted with water and washed with EtO_2. A supernatant oil consisting of the sodium salt of acid **10**, insoluble in the strongly ionic aqueous phase, was separated out from the aqueous phase and dissolved in pure water. This solution was acidified (pH = 1) with concd. aqueous HCl, and the new oil formed was separated out and reprecipitated from EtOH/water to give 34 mg of **10**, 82% pure by HPLC (ca. 37%), noncrystalline solid.– IR (KBr): ν = 3200 cm^{-1} (br., OH), 2240 (w, CN), 1730 (s, C=O).– ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.41 (s, 3 H, 2-CH_3), 1.47 (s, 3 H, 2-CH_3), 3.74 (s, 3 H, OCH_3), 3.88 (s, 3 H, OCH_3), 6.7–7.0 (m, 3 H, aromatic H), 7.2–7.5 (m, 5 H, aromatic H).

*4-Nitrobenzophenone (**11**)*

The procedure for **6a** was followed, using 0.14 g (1.3 mmol) of *t*BuOK in 2 ml of dry DMSO, 300 mg (1.26 mmol) of phenyl-(4-nitrophenyl)acetonitrile in 2 ml of dry DMSO, 185 µl (1.26 mmol) of ethyl bromodimethylacetate and a 4-d reaction time. The reaction mixture was diluted with water, and a precipitate was filtered out and washed with water to give 277 mg of **11** (97%), mp 131–137 °C (ref.[24] 138 °C); IR and ^{1}H NMR spectra in agreement with structure **11**.

*Ethyl 2-Methyl-3-cyano-3-phenyl-3-(4-nitrophenyl)propionate (**12**)*

The procedure for **6a** was followed, using 1.41 g (12.6 mmol) of *t*BuOK in 30 ml of dry DMSO, 3.01 g (12.6 mmol) of phenyl-(4-nitrophenyl)acetonitrile in 10 ml of dry DMSO, 1.6 ml (12 mmol) of ethyl 2-bromo-2-methylacetate and a 3-d reaction time. Kugelrohr distillation of the crude product (oven temp. 200–210 °C/0.2 Torr) gave 1.33 g of **12** (31%), viscous liquid.– ^{1}H NMR (200 MHz, $CDCl_3$; diastereomeric ratio = 1:1): δ = 1.06 and 1.10 (2 t, *J* = 7 Hz, 3 H, OCH_2CH_3); 1.40 and 1.47 (2 d, *J* = 7 Hz, 3 H, 2-CH_3); 3.65 and 3.68 (2 q, *J* = 7 Hz, 1 H, 2-H); 4.04 (q, *J* = 7 Hz, 2 H, OCH_2CH_3); 7.3–7.5 (m, 5 H, aromatic H); 7.35 and 7.69 (2 d, *J* = 9 Hz, 2 H,aromatic H); 8.19 and 8.22 (2 d, *J* = 9 Hz, 2 H, aromatic H).– Anal. ($C_{19}H_{18}N_2O_4$) C, H, N.

Kugelrohr distillation gave an early solid fraction (oven temp. 130–180 °C/0.5 Torr) which was recrystallized from EtO_2/CH_2Cl_2 to yield 1.01 g of 4-nitrobenzophenone (**11**, 35%), mp 134–138 °C; R_f identical with that of 4-nitrobenzophenone above.

*Ethyl 2,2-Dimethyl-3-cyano-3-phenyl-3-(4-nitrophenyl)propionate (**9d**)*

To solution of 3.6 mmol of *n*BuLi in THF/*n*-C_6H_{14} at 0 °C under nitrogen (prepared from 1.8 ml of a 2.0 M solution of *n*BuLi in *n*-C_6H_{14}, and 3 ml of dry THF), 0.50 ml (3.6 mmol) of *i*Pr_2NH (distilled from KOH) was added dropwise with stirring, followed by dropwise addition of a solution of 1.20 g (3.60 mmol) of **12** in 3 ml of dry THF and, then, by addition of 0.75 ml (12 mmol) of MeI. After 3 d, the mixture was diluted with water and extracted with Et_2O, and the ethereal phase was washed with water and dried (Na_2SO_4). Separation by TLC on silica gel with petroleum ether/EtO_2 (4:1) as mobile phase (R_f = 0.2) gave 98 mg of **9d** (8%), mp 102–107 °C.– ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.22 (t, *J* = 7 Hz, 3 H, OCH_2CH_3), 1.81 (s, 3 H, 2-CH_3), 1.94 (s, 3 H, 2-CH_3), 4.25 (m, 2 H, OCH_2CH_3), 7.0–7.4 (m, 7 H, aromatic H), 8.11 (d, *J* = 9 Hz, aromatic H).– Anal. ($C_{20}H_{20}N_2O_4$) N; > 99.0% pure by HPLC.

*2,2-Dimethyl-3-cyano-3,3-diphenyl-N-methyl-N-(3,4-dimethoxyphenethyl)-propionamide (**4a**)*

To a solution of 1.2 mmol of *n*BuLi in THF/*n*-C_6H_{14} at 0 °C under nitrogen (prepared from 0.60 ml of a 2.0 M solution of *n*BuLi in *n*-C_6H_{14}, and 2 ml of dry THF), 215 µl (1.17 mmol) of distilled 3,4-dimethoxy-*N*-methylphenethylamine was added dropwise with stirring, followed by addition of a solution of 353 mg (1.15 mmol) of **9a** in 2 ml of dry THF. After 1 d at room temp., the mixture was diluted with water and extracted with Et_2O, and the ethereal phase was washed with 1 M aqueous HCl and then with water, and dried (Na_2SO_4). The crude liquid product was digested with 100 ml of petroleum ether by vigorous stirring at room temp. overnight, upon which the product solidified, and the sticky undissolved solid separated out from the supernatant liquid. Recrystallization from Et_2O/CH_2Cl_2 gave 156 mg of **4a** (29%), mp 115–119 °C.– IR (KBr): ν = 2200 cm^{-1} (w, C≡N), 1620 (s, C=O).– ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.56 (s, 6 H, 2-CH_3), 2.80 (t, *J* = 7 Hz, 2 H, NCH_2CH_2Ar), 2.90 (s, 3 H, NCH_3), 3.52 (t, *J* = 7 Hz, 2 H, NCH_2CH_2Ar), 3.87 (s, 6 H, OCH_3), 6.7–6.9 (m, 3 H, aromatic H), 7.2–7.5 (m, 10 H, aromatic H).– ^{13}C NMR (50 MHz, $CDCl_3$): δ = 25.2, 33.0, 37.4, 49.1, 53.2, 56.0, 60.9, 111.4, 112.2, 120.8, 124.0, 127.5, 127.8, 130.0, 131.5, 139.7, 147.7, 149.0, 173.8.– Anal. ($C_{29}H_{32}N_2O_3$) C, H, N.

*2,2-Dimethyl-3-cyano-3-phenyl-3-(3,4-dimethoxyphenyl)-N-methyl-N-(3,4-dimethoxyphenethyl)propionamide (**4b**)*

The procedure for **4a** was followed, using 0.70 ml (1.4 mmol) of the *n*BuLi solution and 2 ml of THF, 255 µl (1.38 mmol) of 3,4-dimethoxy-*N*-methylphenethylamine, 255 mg (0.695 mmol) of **9b** in 2 ml of THF and a 3-h reaction time. The crude liquid product was digested with 100 ml of petroleum ether/EtO_2 (25:1) by vigorous stirring at room temp. overnight, upon which the product solidified, and the sticky undissolved solid was separated out from the supernatant liquid. By two additional digestions at room temp. with 100 ml of petroleum ether and 100 ml of petroleum ether/EtO_2 (25:1) was obtained 50 mg of **4b** (14%), noncrystalline solid. On concentration of the supernatant liquid from the last digestion there precipitated an additional 66 mg of **4b** (19%).– IR (KBr): ν = 2240 cm^{-1} (vw, C≡N), 1640 (C=O).– ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.56 (s, 3 H, 2-CH_3), 1.57 (s, 3 H, 2-CH_3), 2.82 (t, *J* = 8 Hz, 2 H, NCH_2CH_2Ar), 2.85 (s, 3 H, NCH_3), 3.49 (t, *J* = 8 Hz, 2 H, NCH_2CH_2Ar), 3.77 (s, 3 H, OCH_3), 3.86 (s, 9 H, OCH_3), 6.7–7.0 (m, 6 H, aromatic H), 7.2–7.5 (m, 5 H, aromatic H).– ^{13}C NMR (100 MHz, $CDCl_3$): δ = 25.5, 25.6, 33.0, 37.4, 49.4, 53.2, 55.8, 55.9, 55.96, 56.00, 60.6, 110.2, 111.4, 112.1, 113.9, 120.7, 122.4, 123.7, 127.6, 127.9, 129.7, 131.4, 131.8, 139.7, 147.7, 148.3, 148.4, 149.0, 174.0.– Anal. ($C_{31}H_{36}N_2O_5$) C, H, N.

*2,2-Dimethyl-3-cyano-3-isopropyl-3-(3,4-dimethoxyphenyl)-N-methyl-N-(3,4-dimethoxyphenethyl)propionamide (**4c**)*

The procedure for **4a** was followed, using 0.49 ml (0.98 mmol) of the *n*BuLi solution and 2 ml of THF, 190 µl (1.03 mmol) of 3,4-dimethoxy-*N*-methylphenethylamine, 172 mg (0.516 mmol) of **9c** in 2 ml of THF and a 3-h reaction time. By TLC of the crude product on silica gel with C_6H_6/AcOEt (4:1) as mobile phase (R_f = 0.5) was obtained 105 mg of **4c** (42%), noncrystalline solid.– IR (KBr): ν = 2200 cm^{-1} (vw, C≡N), 1600 (C=O).– ^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.77 [d, *J* = 7 Hz, 3 H, CH(CH_3)$_2$], 1.41 [d, *J* = 7 Hz, 3 H, CH(CH_3)$_2$], 1.48 (s, 3 H, 2-CH_3), 1.59 (s, 3 H, 2-CH_3), 2.56 (s, 3 H, NCH_3), 2.93 [sept, *J* = 7 Hz, 1 H, C*H*(CH$_3$)$_2$], 2.61 (t, *J* = 8 Hz, 2 H, NCH$_2$C*H*$_2$Ar), 3.1–3.5 (br., 2 H, NC*H*$_2$CH$_2$Ar), 3.84 (s, 3 H, OCH_3), 3.85 (s, 6 H, OCH_3), 3.86 (s, 3 H, OCH_3), 6.6–6.8 (m, 4 H, aromatic H), 7.0 (br., 2 H, aromatic H).– ^{13}C NMR (125 MHz, $CDCl_3$): δ = 21.3, 22.0, 27.8, 29.7, 32.8 (br.), 34.0, 37.4, 51.7, 53.4 (br.), 55.85, 55.87, 56.0, 60.3, 110.3 (br.), 111.1, 111.8, 120.0 (br.), 120.3, 120.6, 130.4, 131.3, 147.5, 148.5 (br.), 148.8, 174.1.– Anal. ($C_{28}H_{38}N_2O_5$) C, H, N.

*2,2-Dimethyl-3-phenyl-3-(3,4-dimethoxyphenyl)-4-imino-N-(3,4-dimethoxyphenethyl)-γ-butyrolactam (**5a**)*

The procedure for **4a** was followed, using 0.91 ml (1.8 mmol) of the *n*BuLi solution and 2 ml of THF, 293 µl (1.76 mmol) of distilled 3,4-dimethoxyphenethylamine, 309 mg (0.842 mmol) of **9b** in 3 ml of THF, and a 20-h reaction time. The resulting mixture was stirred with 15 ml of 1 M aqueous HCl, and then brought to pH = 8 with 1 M aqueous NaOH, which formed a precipitate. The mixture was extracted with Et_2O, and the ethereal phase was washed with water and dried (Na_2SO_4). TLC of the crude product on silica gel with $C_6H_5CH_3$/AcOEt (3:2) as mobile phase (R_f = 0.4) afforded 253 mg of **5a** (60%), noncrystalline solid.– IR (KBr): ν = 3260 cm^{-1} (m, NH), 1720 (s, C=O), 1630 (s, C=N).– ^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.96 (s, 3 H, 2-CH_3), 1.00 (s, 3 H, 2-CH_3), 2.82 (t, *J* = 8 Hz, 2 H, NCH$_2$C*H*$_2$Ar), 3.73 (s, 3 H, OCH_3), 3.82 (s, 3 H, OCH_3), 3.84 (s, 3 H, OCH_3), 3.87 (s, 3 H, OCH_3), 3.87 (t, *J* = 8 Hz, 2 H, NC*H*$_2$CH$_2$Ar), 6.6–6.9 (m, 6 H, aromatic H), 7.2–7.4 (m, 5 H, aromatic H).– ^{13}C NMR (50 MHz, $CDCl_3$): δ = 23.0, 23.8, 32.1, 40.4, 48.3, 55.8, 55.9, 56.0, 65.0, 110.8, 111.3, 112.3, 112.7, 120.9, 121.6, 127.4, 128.3, 128.9, 130.9, 131.9, 139.8, 147.7, 148.4, 148.7, 148.9, 169.6, 181.4.– Anal. ($C_{30}H_{34}N_2O_5$) C, H, N.

Biological Test

MDR P400 cell subline was isolated from an individual soft-agar clone of L5178Y cells previously adapted to grow in the presence of increasing vincristine concentrations. P400 cells show cross-resistance to a variety of antineoplastic agents, decreased drug accumulation, and overexpression of plasma-membrane P-glycoprotein,[16,17] as typical of MDR cells. Both P400 and L5178Y cells were maintained as suspension cultures in Fischer's medium supplemented with 10% horse serum, and with passage twice a week; the culture medium of P400 cells contained vincristine ($0.1 \times IC_{50}$), which was removed one week before any experimental procedure.

Vincristine was used as the sulfate salt and the standard compound verapamil as the hydrochloride. The compounds **3a**, **3b**, **4a**–**4c**, **5a**, and **5b** were dissolved in DMSO; the final DMSO concentration in the culture medium was 1.4×10^{-4} M.

The cells were incubated in the presence of increasing vincristine concentrations and a constant tested-compound concentration at 37 °C in a humidified 5%-CO_2 atmosphere. After 72 h, the cells were counted using a ZM-Coulter counter. Control cultures (containing the DMSO and neither vincristine nor tested compound) grew logarithmically during this entire period. Vincristine IC_{50} was defined as the vincristine concentration that reduced by 50% the cell growth attained by the control culture, and was determined from a semilogarithmic plot of cell growth vs. vincristine concentration using GraphPad InPlot 3.0.1 program. Relative resistance was expressed as the ratio of vincristine IC_{50} for MDR P400 cells to that for parental L5178Y cells.

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