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Reversal of doxorubicin resistance and catalytic neutralization of lysosomes by a lipophilic imidazole

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Abstract

A number of lipophilic nitrogenous bases, designed to act as membrane-active, catalytic proton transfer agents, were tested for their ability to neutralize the acidity of lysosomes, a model for other acidic intracellular vesicles involved in drug sorting. The most successful of these, an imidazole **1**, caused a 1.7 unit rise in lysosomal pH of RAW cells at 100 μM, compared to a 0.2 and 1.4 unit rise for ammonium chloride at 100 μM and 10 mM, respectively. Compound **1** also exhibited potent reversal of doxorubicin (DOX) resistance in the HCT116-VM46 cell line by a factor of 14 over the sensitive strain, and superior to that of widely used verapamil (VRP) by a factor of 1.75 at 20 μM. It also has antiviral properties, and potential applications in other lysosome-related areas such as immunotoxin potentiation and the control of bacterial toxins, immune response, prion replication, malaria and intralysosomal microorganisms.

Key words: Multidrug resistance; MDR; Lysosome; Lysosomotropic; Antiviral

1. Introduction

The survival of multidrug resistant cancer cell populations following chemotherapy and their subsequent growth into tumors that no longer respond to continued therapy is one of the primary difficulties in cancer treatment [1]. Cells that acquire resistance to a single agent such as doxorubicin are then usually resistant to a wide range of other, structurally unrelated drugs such as vinca alkaloids, mitomycins, etoposide and taxol. Multidrug resistance (MDR) has been linked to the expression of P-glycoprotein (PGP), a 170 kDa protein that acts as a transmembrane, ATP-dependent exporter of drugs [2]. However, a number of recent studies have demonstrated MDR in cells containing no detectable levels of PGP, and there is a consensus that PGP cannot be the only factor involved in MDR [3–5].

There is increasing evidence that MDR cells are able to compartmentalize in acidic vesicles drugs that have entered the cytosol, thereby isolating them from their site of action, usually the nucleus [6,7]. The drug is then expelled, either by PGP or in some other way such as exocytosis.

Many of the agents that have been shown to be effective at reversing MDR have two common features: they contain at least one basic nitrogen and they are lipophilic [8]. Verapamil (VRP) and chloroquine (CQ) (Fig. 1) are two of the best known examples. Although the mechanism of action of verapamil has been proposed to arise from its binding to PGP, presumably blocking drug efflux directly [9], there has been some speculation that another effect of MDR reversing drugs may be to raise the pH of acidic vesicles, perhaps altering the pattern of drug sorting or compartmentalization so that the antitumor drugs become less accessible to PGP for expulsion from the cell [10–15]. The former interpretation is weakened and the latter supported by the fact that both enantiomers of VRP are equally active against MDR [16–22], and that MDR reversal is not always correlated with photoaffinity labeling of PGP [23–25], which suggests that association of VRP with PGP, an optically active protein, is

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Abbreviations: CQ, chloroquine; DOX, doxorubicin; FITC, fluorescein isothiocyanate; IT, immunotoxin; MDR, multidrug resistance; MHC, major histocompatability complex; PGP, P-glycoprotein; PrP, prion protein; SFV, Semliki forest virus; VRP, verapamil; VSV, vesicular stomatitis virus.

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not obligatory for MDR. Transmembrane proton transport, in contrast, is not expected to depend on optical activity.

The best model for intracellular acidic vesicles are lysosomes. Nitrogenous bases such as ammonium chloride and methylamine, which raise lysosomal pH, are well known through the work of Ohkuma and Poole [26]. Most of these agents cause vacuolization of lysosomes as a result of a buildup of osmotic stress. This is because the unprotonated bases pass easily into lysosomes through their membranes, but cannot readily depart because, after protonation, they strongly prefer to remain in the aqueous phase, so that the phospholipid membrane now becomes a barrier. An exception was tributylamine which, Poole and Ohkuma noted [27], may be lipophilic enough to cross freely even when protonated. They also observed that CQ was effective at much lower (μM) concentrations but offered no mechanistic explanation.

One effect that we thought tributylamine, VRP, and perhaps CQ might have, to some extent, is the neutralization of intracellular acidic compartments through catalytic proton transport to the cytosol (Fig. 2). According to this mechanism the lipophilic amine localizes within the hydrocarbon zone of the acidic vesicle's phospholipid membrane, becomes protonated on the interior side, and loses its proton on the cytosolic side, all the while remaining in the membrane. The driving force is dissipation of the pH gradient on either side of the membrane.

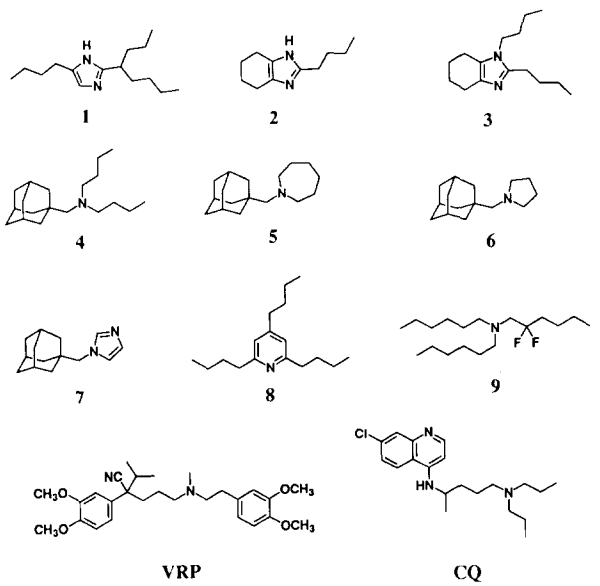

Fig. 1. Structures of the compounds used in this study: **1**, 2-(1-propyl)pentyl-5-butylimidazole; **2**, 2-butyl-5,6-cyclohexylimidazole; **3**, 1,2-dibutyl-5,6-cyclohexylimidazole; **4**, *N,N*-dibutyladamantylmethylamine; **5**, *N*-adamantylmethylazacycloheptane; **6**, *N*-adamantylmethylpyrrolidine; **7**, *N*-adamantylmethylimidazole; **8**, 2,4,6-tributylpyridine; **9**, *N,N*-dihexyl-2,2-difluorohexylamine; VRP, verapamil; CQ, chloroquine.

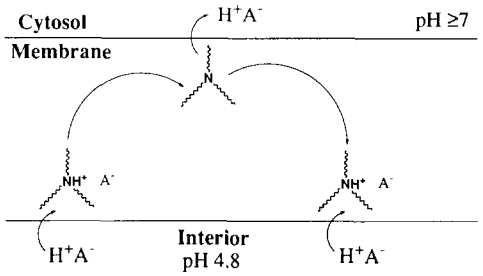

Fig. 2. Schematic model of lysosomal neutralization by a membrane-active proton transporter.

A drawback to the use of VRP in the clinic, as well as most of the drugs that have shown reversal of MDR in vitro, has been toxicity. This is because most of these agents, which were developed to treat other conditions, exhibit potent activity in other physiological systems at or below concentrations that make them useful MDR modulators. We sought to prepare nontoxic agents designed specifically to carry out the neutralization of acidic vesicles (lysosomes and endosomes) by the mechanism described above. In order for the compounds to localize in the phospholipid membrane without disrupting it the polar, basic portion of the molecule had to be centered between at least three lipophilic groups. Having only one lipophilic group would make the molecule a detergent, which would disrupt the membrane; having two groups would make it a bilayer former and thus very slow to move from one side of the membrane to the other. Our experience with lysosomotropic detergents suggested that the basic portion of the molecule should have a pK_a between 5 and 7 so that it would selectively localize in lysosomal membranes [28,29].

2. Materials and methods

Verapamil, doxorubicin, chloroquine and fluorescein isothiocyanate dextran (FITC dextran) were purchased from Sigma (St. Louis, MO). Tributylamine, methylamine hydrochloride and ammonium chloride were purchased from Aldrich Chemical (Milwaukee, WI). Molecusol® was purchased from Pharmatec (Alachua, FL). The synthesis of lysosomotropic agents **1–9** will be described separately [30]. Culture medium was purchased from Gibco (Grand Island, NY). Cell lines (RAW and HCT116-VM46) were obtained from the American Type Culture Collection.

Molecusol complex of imidazole 1

Compounds **1–9** are too lipophilic for dispersal in aqueous medium. However, **1** fits into the hydrophobic cavity of molecusol (3-hydroxypropylated cyclodextrin), a nontoxic, water-soluble dispersing agent. Thus **1**

(about 100 μmol) was dissolved in absolute ethanol (0.2 ml) in a glass vessel, and treated with 0.40 ml (about 1.25 equivalents) of a 37% w/v solution of molecusol in water. The solvents were evaporated by passing a stream of nitrogen over the stirred mixture at room temperature. When only a glassy solid remained, residual solvent was removed in vacuo (about 0.05 mmHg) over 24 h. The solid was dissolved in water to give a final concentration of 10 mM. Binding of **1** to molecusol was confirmed by NMR spectroscopy and is tight enough for it to remain in aqueous solution, but not too tight to prevent it from entering cells.

In vitro lysosomal neutralization

RAW cells were incubated with fluorescein isothiocyanate dextran (FITC-dextran) [26] (final concn. 5 mg/ml), which concentrates selectively in lysosomes, for 24 h at 37°C. They were then spun down, washed four times with RPMI medium (without serum or glutamine), and diluted to $1 \cdot 10^7$ cells/ml in RPMI. $1 \cdot 10^6$ cells were removed, diluted to 1 ml with RPMI and loaded on the flow cytometer (Epics V made by Coulter Electronics). The sample cell was excited at 488 nm and emission measured at 525 nm with a band pass of ±5 nm. The amplitude of emission of fluorescein increases proportionally with pH over the range of interest. A baseline pH (about 4.8) was measured and the compound of interest was added. The change in lysosomal pH was measured over time. Ammonium chloride at 10 mM, which has been shown to raise lysosomal pH to about 6.2 [26], was used as a standard. The viability of treated cells was evaluated by monitoring the forward angle light scatter and the 90° angle light scatter in the flow cytometer.

In vitro reversal of multidrug resistance

A DOX-resistant human colon carcinoma cell line, HCT116-VM46, was grown in RPMI medium at 37°C in 5% CO_2, and incubated with DOX (2 μg/ml) alone or with either VRP or **1**, at 50 μM and 20 μM, for 1 h at 25°C in a glass vessel. $1 \cdot 10^6$ cells were removed, diluted to 1 ml with RPMI and loaded on the flow cytometer (Epics V made by Coulter Electronics). Histograms representing intracellular accumulation of DOX in viable cells were recorded by exciting the sample cell at 488 nm and measuring DOX fluorescence at 570 nm. For comparison, the parental cell line, HCT116, was incubated with 2 μg/ml DOX and its intracellular drug retention measured in the same way.

3. Results and discussion

We prepared several series of compounds centered around imidazole, pyridine, and 2,2-difluoroethylamine, as well as normal trialkylamines. Since the molecules had to reside in the bilayer membrane, they needed sufficient hydrocarbon mass to remain in an organic phase in preference to water, even while completely protonated. We also had to concern ourselves with delivery of these agents to cells, so that a practical balance had to be struck between lipophilicity and aqueous solubility. We carried out pK_a studies in chloroform, an acceptable surrogate for the hydrophobic zone of natural bilayer membranes. This was done by measuring the proton NMR chemical shifts upon protonation after stirring in a two-phase $CDCl_3$/phosphate buffer system with the pH of the buffer incrementally varied from 2 to 10. The phosphate ion is not easily carried into chloroform, even by a very lipophilic cation, so the studies could be confidently carried out in the presence of other anions of interest. The pK_a values measured in this way (Table 1) are generally lower than those in a single aqueous phase owing to the resistance of the anion to enter the organic phase, and are thus anion dependent. The results shown in Table 1 are for the cases where chloride served as the anion (Dubowchik, G.M. et al., in preparation). For each compound type, hydrocarbon mass was gradually added until extraction from chloroform into aqueous acid did not occur.

Table 1
RAW cell lysosomal neutralization induced by lysosomotropic amines

Compound	pK_a	Concn. (mM)	Vehicle	ΔpH
NH_4Cl	9.21 [a] [41]	10	water	1.4
NH_4Cl	9.21 [a] [41]	1	water	0.7
NH_4Cl	9.21 [a] [41]	0.1	water	0.2
CH_3NH_3Cl	10.62 [a] [41]	10	water	1.4
Chloroquine	10.1 [a] [42]	0.1	water	0.2
Tributylamine	10.89 [a] [41]	10	water	0
1	5.5	0.1	lipid emulsion	0.5
1·HCl	5.5	0.1	water	1.0
1·HCl	5.5	0.1	molecusol complex [b]	1.7
2·HCl	N.T.	1	water	0.4
3·HCl	N.T.	1	water	0.6
4·HCl	5.3	0.1	water	0.2
4	5.3	1	lipid emulsion	0.3
5	5.5	1	lipid emulsion	< 0.1
6	6.1	1	lipid emulsion	< 0.1
7	– [c]	1	lipid emulsion	< 0.1
8	3.2	0.1	lipid emulsion	0.2
9	1.7	1	lipid emulsion	< 0.1

RAW cells were allowed to endocytose fluorescinated dextran (FITC-dextran) (final concn. 5 mg/ml) for 24 h at 37°C. They were then spun down, washed four times with RPMI medium, and then diluted to $1 \cdot 10^7$ cells/ml in RPMI. $1 \cdot 10^6$ cells were removed, diluted to 1 ml with RPMI and loaded on the flow cytometer. The sample cell was excited at 488 nm and emission was measured at 525 nm. A baseline pH (about 4.8) was measured and the compound of interest was added. The change in lysosomal pH was monitored over time and in all cases remained constant after about 15 min.

[a] Measured in a single phase aqueous system.

[b] Molecusol alone had no effect on the pH of the cells and was nontoxic.

[c] Extracted into aqueous acid.

The compounds shown in Fig. 1 met the criteria of appropriate pK_a and lipophilicity, and were therefore chosen to test for lysosomal neutralization activity. The best of these, imidazole **1**, was then tested in an MDR assay measuring cellular accumulation of doxorubicin (DOX).

Modification of lysosomal pH has also been shown to be important in other biological processes that are therapeutically relevant. One such process that we briefly examined is viral uncoating, which many viruses must undergo in an acidic milieu such as lysosomes or endosomes in order to replicate [31]. Substances such as lysosomotropic amines or ionophores that raise lysosomal pH have been shown to be effective against influenza [31,32], herpes [31,33], SFV [31,34], VSV [31,35], paramyxovirus [31], rabies [36–38], hoof-and-mouth [39], and hepatitis A [40]. Compound **1** was tested in our influenza assay.

Compounds **1–9** were tested for their ability to cause lysosomal neutralization of RAW cells over time by measurement of the fluorescence emission of FITC-dextran using a flow cytometer (Table 1). RAW cells are a murine monocytic macrophage line that are rich in lysosomes, established from an ascites of a tumor in a male mouse by intraperitoneal injection of Abelson leukemia virus. Upon incubation, the cells selectively localize FITC-dextran in lysosomes by endocytosis. The degree of neutralization over time differed greatly depending on the compound. As observed by Ohkuma and Poole [26] ammonium chloride caused an almost instantaneous rise in pH. We have used their standard of pH 6.2 at 10 mM ammonium chloride as the yardstick by which we measured the pH differences induced by our new compounds. Methylamine hydrochloride, also at 10 mM, raised the pH to the same degree but over several minutes. We were unable, however, to duplicate their results with tributylamine (using no dispersing agent) and CQ. Tributylamine was not very soluble in the medium and little may have reached the cells. We have no explanation for the failure of CQ at 100 μM to match the activity of ammonium chloride at 10 mM. Our compounds caused a steady rise in lysosomal pH over about 15 min at which time it leveled off.

All of the compounds exhibited some degree of activity and were nontoxic to the cells at the concentrations tested. The viability of the cells was assured by gating the detector so that dead or dying cells would appear as a separate population as indicated by measuring forward angle light scatter and 90° angle light scatter. Being very lipophilic, they would not simply dissolve unaided in medium. At first we used a milky emulsion formed by sonicating the compounds with egg yolk phosphatidylcholine in medium. This was sufficient to determine which compounds looked promising but was hardly satisfactory. Imidazole **1** was clearly superior in these initial tests even when it was obvious that much of the compound was not reaching the cells. At 1 mM most of the other compounds showed detectable but very slight neutralization. Exceptions were the tributylpyridine **8**, and adamantylmethyldibutylamine **4**. Neither retained enough activity in subsequent tests to warrant continued interest.

It became clear to us in the course of our studies that these compounds displayed much less activity when assays were done using plastic, presumably because of binding of the lipophilic compounds to the walls of the vessel. Therefore, whenever possible, biological testing was done in glass vessels.

We found that delivery of **1** to the cells could be improved greatly, first by forming the hydrochloride salt and adding it to the cells as a water solution, thereby doubling the lysosomal pH increase, and then by forming the molecusol complex of the hydrochloride. This was completely water soluble to a concentration of **1** of at least 10 mM. At 100 μM, **1** raises lysosomal pH to about 6.5 within 15 min as its molecusol complex. This is superior to ammonium chloride at 100 times the concentration and, taken together with the fact that the hydrochloride salt of **1** is not extracted out of chloroform into water, argues strongly for its acting by the mechanism proposed above. The fused cyclohexylimidazoles **2** and **3** also exhibited activity that was significant, but still greatly inferior to that of **1**.

The MDR reversing activity of **1** was next compared with VRP in combination with doxorubicin (DOX) in a DOX-resistant strain (VM46) of the HCT116 human colon cancer cell line. The VM46 strain is 7-fold resistant to DOX in comparison with the sensitive strain. At 20 μM (5.43 μg/ml) **1** induced a 14-fold increase in retention of DOX by the resistant strain as compared with an 8-fold increase at the same concentration of VRP (9.1 μg/ml) (Fig. 3). Thus **1** caused the resistant line to retain twice the amount of DOX as the sensitive line. At 50 μM (13.6 μg/ml) **1** induced a 17-fold increase in retention of DOX by the resistant line, almost 2.5-times the level of the sensitive line. Imidazole **1**, therefore, was superior to VRP by a factor of 1.75 in the HCT116 cell line.

The molecusol complex of **1** also exhibited activity against the influenza virus in vitro. After incubation of MDCK (Madin-Darby canine kidney) cells with virus for one hour the compound was added. After 4–5 days the number of plaques were counted. The ED_{50} (the concentration that reduces plaque formation 50% in relation to untreated control cells) of **1** for plaque reduction was 48 μM (13.3 μg/ml). Because the assay was done in plastic **1** would most likely be even more effective in glass.

In conclusion, we have prepared agents specifically designed to neutralize the pH of lysosomes by localizing in the lysosomal membrane and exporting protons

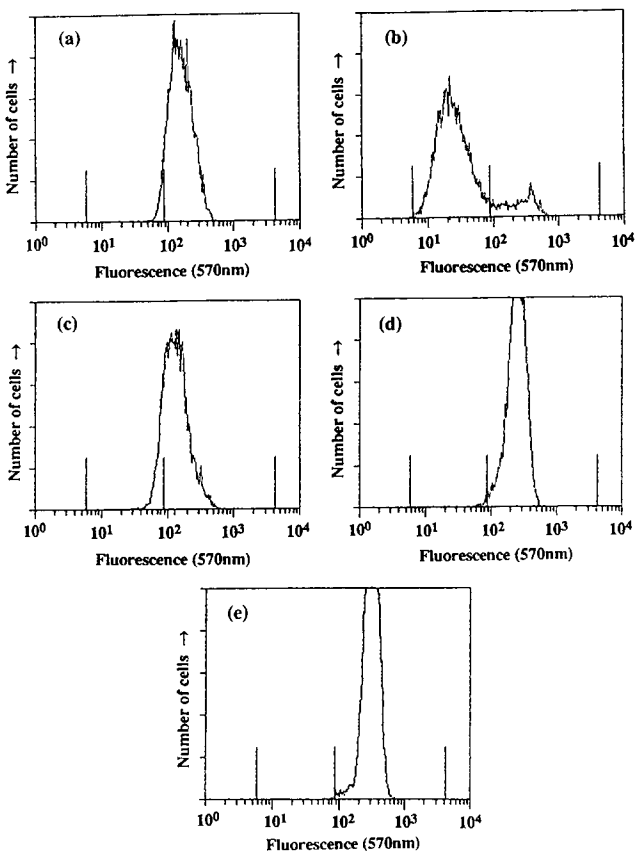

Fig. 3. Histograms showing DOX accumulation in the parental and resistant (VM46) strains of the HCT116 human colon carcinoma cell line after incubation at room temperature with 2 μg/ml DOX, alone and with either VRP or **1**. (a) HCT116 DOX-sensitive cell line; (b) HCT116-VM46 DOX-resistant cell line; (c) HCT116-VM46 with 20 μM VRP; (d) HCT116-VM46 with 20 μM **1**; (e) HCT116-VM46 with 50 μM **1**.

catalytically. The most successful of these, imidazole **1**, is more effective at 100 μM than ammonium chloride at 10 mM, strongly suggesting that it is working in this way. Compound **1** was more effective than VRP at reversing DOX resistance in the HCT116-VM46 cell line by a factor of 1.75 at a concentration of 20 μM (5.43 μg/ml). Replication of influenza virus was also inhibited by **1** ($ED_{50} = 48$ μM (13.3 μg/ml)). We believe that these activities, taken together, favor the proposed mechanism, and that further design and testing may result in even more effective agents which work in this way.

4. Potential applications

There are other disease states where efficient lysosomotropic amines might be useful. (1) Immunotoxins (ITs) can be potentiated by retarding intralysosomal proteolysis [43] through control of lysosomal pH [44–46]. Indeed, VRP, the prototypical anti-MDR amine, does potentiate ITs [47]. (2) Many bacterial toxins translocate into the cytosol from intracellular vesicles only at low pH [48,49], a process inhibited for diphtheria, botulinum and cholera toxins by lysosomotropic amines and monensin [50–53]. (3) Antigen (Ag)-presenting cells process Ag by proteolysis in lysosomes or endosomes [43,54], processed Ag binds MHC class I compounds at low pH [55], and presentation is inhibited by lysosomotropic amines [54]. (4) Lysosomes may play a key role in prion diseases, acting as a 'bioreactor' for conversion of normal PrP^C to the scrapie agent PrP^{SC} which then induces release of enzymes that cause neuronal cytoskeletal disruption [56]. (5) The malaria parasite, *Plasmodium falciparum*, has an acidic digestive vacuole whose activity is essential for its viability [57]. (6) Numerous pathogenic microorganisms live and even replicate within lysosomes, where they are shielded from the body's immune system and from antibiotics [58]. In some cases, raising lysosomal pH is known to interfere with their life cycle (legionella, *Trypanosoma cruzi*) [59,60].

5. Acknowledgments

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6. References

[1] Harris, A.L. and Hochhauser, D. (1992) Acta Oncol. 31, 205–213.
[2] Spoelstra, E.C., Westerhoff, H.V., Dekker, H. and Lankelma, J. (1992) Eur. J. Biochem. 207, 567–579.
[3] Marquardt, D. and Center, M.S. (1992) Cancer. Res. 52, 3157–3163.
[4] Versantvoort, C.H.M., Broxterman, H.J., Pinedo, H.M., Devries, E.G.E., Feller, N., Kuiper, C.M. and Lankelma, J. (1992) Cancer Res. 52, 17–23.
[5] Huet, S., Schott, B. and Robert, J. (1992) Br. J. Cancer 65, 538–544.
[6] Boiocchi, M. and Toffoli, G. (1992) Eur. J. Cancer 28A, 1099–1105.
[7] Slapak, C.A., Lecerf, J.M., Daniel, J.C. and Levy, S.B. (1992) J. Biol. Chem. 267, 10638–10644.
[8] Hofsli, E. and Nissen-Meyer, J. (1990) Cancer Res. 50, 3997–4002.
[9] Cornwell, M.M., Pastan, I. and Gottesman, M.M. (1987) J. Biol. Chem. 262, 2166–2170.
[10] Zamora, J.M., Pearce, H.L. and Beck, W.T. (1988) Mol. Pharmacol. 33, 454–462.
[11] Klugmann, F.B., Decorti, G., Crevellato, E., Candussio, L., Mallardi, F. and Baldini, L. (1990) Anticancer Res. 10, 1571–1578.
[12] Klohs, W.D. and Steinkampf, R.W. (1988) Mol. Pharmacol. 34, 180–185.
[13] Shiraishi, N., Akiyama, S.-I., Kobayashi, M. and Kuwano, M. (1986) Cancer Lett. 30, 251–259.

[14] Zamora, J.M. and Beck, W.T. (1986) Biochem. Pharmacol. 35, 4303–4310.
[15] Sehested, M., Skovsgaard, T. and Roed, H. (1988) Biochem. Pharmacol. 37, 3305–3310.
[16] Plumb, J.A., Milroy, R. and Kaye, S.B. (1990) Biochem. Pharmacol. 39, 787–792.
[17] Pirker, R., Keilhauer, G., Raschack, M., Lechner, C. and Ludwig, H. (1990) Int. J. Cancer, 45, 916–919.
[18] Höllt, V., Kouba, M., Dietel, M. and Vogt, G. (1992) Biochem. Pharmacol. 43, 2601–2608.
[19] Häussermann, K., Benz, B., Gekeler, V., Schumacher, K. and Eichelbaum, M. (1991) Eur. J. Clin. Pharmacol. 40, 53–59.
[20] Dorr, R.T. and Liddil, J.D. (1991) Cancer Chemother. Pharmacol. 27, 290–294.
[21] Pommerenke, E.W., Mattern, J., Traugott, U. and Volm, M. (1991) Arzeim.-Forsch./Drug Res. 41, 855–858.
[22] Gruber, A., Peterson, C. and Reizenstein, P. (1988) Int. J. Cancer 41, 224–226.
[23] Jaffrézou, J.-P., Herbert, J.-M., Levade, T., Gau, M.-N., Chatelain, P. and Laurent, G. (1991) J. Biol. Chem. 29, 19858–19864.
[24] Sato, S.-i., Watanabe, Y., Okamura, K., Hamanaka, R., Mori, T., Kohno, K. and Kuwano, M. (1989) Anti-Cancer Drug Design 4, 125–135.
[25] Beck, W.T., Cirtain, M.C., Glover, C.J., Felsted, R.L. and Safa, A.R. (1988) Biochem. Biophys. Res. Commun. 159, 959–966.
[26] Ohkuma, S. and Poole, B. (1978) Proc. Natl. Acad. Sci. USA 75, 3327–3331.
[27] Poole, B. and Ohkuma, S. (1981) J. Cell Biol. 90, 665–669.
[28] Miller, D.K., Griffiths, E., Lenard, J. and Firestone, R.A. (1983) J. Cell Biol. 97, 1841–1851.
[29] Firestone, R.A., Pisano, J.M. and Bonney, R.J. (1979) J. Med. Chem. 22, 1130–1133.
[30] Dubowchik, G.M., Padilla, L. and Firestone, R.A. (1994) in preparation.
[31] Marsh, M. (1984) Biochem. J. 218, 1–10.
[32] Stegmann, T., Morselt, H.W.M., Scholma, J. and Wilschut, J. (1987) Biochim. Biophys. Acta 925, 166–170.
[33] Koyama, A.H. and Uchida, T. (1989) Virus Res. 13, 271–282.
[34] White, J., Kartenbeck, J. and Helenius, A. (1980) J. Cell Biol. 87, 264–272.
[35] Miller, D.K. and Lenard, J. (1980) J. Cell. Biol. 84, 430–7.
[36] Tsiang, H. and Superti, F. (1984) Arch. Virol. 81, 377–382.
[37] Superti, F., Derer, M. and Tsiang, H. (1984) J. Gen. Virol. 65, 781–789.
[38] Carrillo, E.C., Giachetti, C. and Campos, R. (1985) Virology 147, 118–125.
[39] Baxt, B. (1987) Virus Res. 7, 257–271.
[40] Superti, F., Seganti, L., Orsi. N., Divizia, M., Gabrielli, R. and Pana, A. (1987) Arch. Virol. 96, 289–296.
[41] Hall, H.K. (1957) J. Am. Chem. Soc. 79, 5441–5444.
[42] De Duve, C., De Barsy, T., Poole, B., Trouet, A., Tulkens, P. and Van Hoof, F. (1974) Biochem. Pharmacol. 23, 2495–2531.
[43] Seglen, P.O., Grinde, B. and Solheim, A.E. (1979) Eur. J. Biochem. 95, 215–225.
[44] Casellas, P., Ravel, S., Bourrie, B.J.P., Derocq, J.-M., Jansen, F.K., Laurent, G. and Gros, P. (1988) Blood 72, 1197–1202.
[45] Jaffrezou, J.-P., Levade, T., Thurneyssen, O., Chiron, M., Bordier, C., Attal, M., Chatelain, P. and Laurent, G. (1992) Cancer Res. 52, 1352–1359.
[46] Ramakrishnan, S., Bjorn, M.J. and Houston, L.L. (1989) Cancer Res. 49, 613–617.
[47] Pirker, R., Fitzgerald, D.J.P., Raschack, M., Zimmerman, F., Willingham, M.C. and Pastan, I. (1989) Cancer Res. 49, 4791–4795.
[48] Fedde, K.N. and Sly, W.S. (1988) J. Cell. Biochem. 37, 233–241.
[49] Montecucco, C., Schiavo, G. and Dasgupta, B.R. (1989) Biochem. J. 259, 47–53.
[50] Marnell, M.H., Stookey, M. and Draper, R.K. (1982) J. Cell Biol. 93, 57–62.
[51] Sandvig, K. and Olsnes, S. (1980) J. Cell Biol. 87, 828–832.
[52] Donovan, J.J. and Middlebrook, J.L. (1986) Biochem. 25, 2872–2876.
[53] Janicot, M., Clot, J.-P. and Desbuquois, B. (1988) Biochem. J. 253, 735–743.
[54] Unanue, E.R. and Allen, P.M. (1987) Science 236, 551–557.
[55] Mouritsen, S., Hansen, A.S., Petersen, B.L. and Buus, S. (1992) J. Immunol. 148, 1438–1444.
[56] Laszlo, L., Lowe, J., Self, T., Kenwards, N., Landon, M., McBride, T., Farquhar, C., McConnell, I., Brown, J., Hope, J. and Mayer, R.J. (1992) J. Pathol. 166, 333–341.
[57] Krogstad, D.J., Schlesinger, P.H. and Gluzman, I.Y. (1985) J. Cell. Biol. 101, 2302–2309.
[58] Boukhala, Z., Renard, C., Baurain, R., Marchand-Brynaert, J., Ghosez, L. and Tulkens, P. (1988) J. Med. Chem. 31, 976–983, and references therein.
[59] Byrd, T.F. and Horwitz, M.A. (1991) J. Clin. Invest. 88, 351–357.
[60] Ley, V., Robbins, E.S., Nussenzweig, V. and Andrews, N.W. (1990) J. Exp. Med. 171, 401–413.