Published in final edited form as:
J Med Chem. 2008 August 14; 51(15): 4563–4570. doi:10.1021/jm800194k.

Antitumor Activity of Bis-Indole Derivatives

Aldo Andreani[*,a], **Silvia Burnelli**[a], **Massimiliano Granaiola**[a], **Alberto Leoni**[a], **Alessandra Locatelli**[a], **Rita Morigi**[a], **Mirella Rambaldi**[a], **Lucilla Varoli**[a], **Laura Landi**[b], **Cecilia Prata**[b], **Michael V. Berridge**[c], **Carole Grasso**[c], **Heinz-Herbert Fiebig**[d], **Gerhard Kelter**[d], **Angelika M. Burger**[e], and **Mark W. Kunkel**[f]

[a]Dipartimento di Scienze Farmaceutiche, Università di Bologna, Via Belmeloro 6, 40126 Bologna, Italy [b]Dipartimento di Biochimica "G. Moruzzi", Università di Bologna, Via Irnerio 48, 40126 Bologna, Italy [c]Malaghan Institute of Medical Research, PO Box 7060, Wellington, New Zealand [d]Oncotest GmbH, Am Flughafen 12-14, 79108 Freiburg, Germany [e]Department of Pharmacology and Experimental Therapeutics, University of Maryland School of Medicine, Marlene and Stewart Greenebaum Cancer Center Bressler Research Building, Rm 9-039, 655 West Baltimore Street, Baltimore, Maryland 21201, USA [f]National Cancer Institute, Division of Cancer Treatment and Diagnosis, Developmental Therapeutics Program, Information Technology Branch, 6130 Executive Blvd., Room 8008, Rockville, Maryland, 20892-7444, USA

Abstract

This paper reports the synthesis of compounds formed by two indole systems separated by a heterocycle (pyridine or piperazine). As a primary screening, the new compounds were submitted to the National Cancer Institute for evaluation of antitumor activity in the human cell line screen. The pyridine derivatives were far more active than the piperazine derivatives. For the study of the mechanism of action, the most active compounds were subjected to COMPARE analysis and to further biological tests including proteasome inhibition and inhibition of plasma membrane electron transport. The compound bearing the 5-methoxy-2-indolinone moiety was subjected to the first *in vivo* experiment (hollow fiber assay) and was active. It was therefore selected for the second *in vivo* experiment (human tumor xenograft in mice).

In conclusion we demonstrated that this approach was successful since some of the compounds described are much more active than the numerous, so far prepared and tested 3-indolylmethylene-2-indolinones.

Introduction

In 1984[2] we published the first of a series of papers devoted to the synthesis of compounds with positive inotropic activity. In 1990[3] we introduced in this project new derivatives arising from the Knoevenagel reaction between an indole aldehyde and a compound containing an active methylene group. Later we found that analogous compounds showed antitumor activity too. In our first paper on 3-(2-chloro-3-indolylmethylene)1,3-dihydroindol-2-ones as antitumor agents,[4] we acknowledged that the first synthesis of the unsubstituted 3-indolylmethylene-1,3-dihydroindol-2-one was performed by a German team in 1969[5] and the first attempt to search an antitumor potential in this class of compounds was made in 1977.[6] We also reported that a team at Farmitalia-Carlo Erba was working on the antitumor activity of analog compounds.[7] In the subsequent years we developed our project based on 3-(2-chloro-3-indolylmethylene)1,3-dihydroindol-2-ones[8–11] whereas the project at Farmitalia-

*Corresponding author: tel +39-051-2099714, fax +39-051-2099734, e-mail aldo.andreani@unibo.it.

Carlo Erba, passing through Pharmacia, Upjohn, Sugen and finally Pfizer, led to Sutent (see Chart 1 and http://www.sutent.com/) which was approved by FDA in 2006.

We also prepared compounds similar to 3-(2-chloro-3-indolylmethylene)1,3-dihydroindol-2-ones but with the two indole systems separated by an heterocycle instead of a methine bridge. These compounds were first tested as positive inotropic agents[12] and later as antitumor agents but only on HeLa cells.[13] In this paper we describe several aspects of the antitumor activity of this class of compounds, reported in Scheme 1, whose structures show similarity to TAS-202[14] and RITA[15] (Chart 1) for their molecular symmetry.

Chemistry

The Knoevenagel reaction we employed in the previous papers involved the condensation of a carbonyl group with a methylene activated by a neighboring electron withdrawing group. The two classes of compounds reported in Scheme 1 present our continued synthetic efforts using the Knoevenagel reaction. In the first case (**3a**–**q**) the carbonyl groups were in the core (a pyridine ring) whereas the active methylene groups were in the indole wings. In the second case (**7**–**9**) this situation was inverted: the active methylene groups were in the core (a piperazine ring) whereas the carbonyl groups were in the indole wings. Moreover the core may be connected to the wings by a double bond (**7a**–**b**) or through a methine bridge (**8a**–**b**, **9a**–**b**), as in compounds **3a**–**q**.

Most of the new derivatives **3** have been prepared with the same procedure described for the previously published compounds:[12,13] the appropriate indolinone **1** in methanol has been treated with pyridine-2,6-dicarbaldehyde **2** in the presence of piperidine (see Scheme 1 and Table 1, method 1). Only compounds **3f**, **3i**–**k** have been prepared in a mixture of acetic acid/hydrochloric acid (method 2).

For the synthesis of compound **3k**, ethyl 2-oxoindoline-5-sulfonate was the starting indolinone. It was prepared by treating 2-oxoindoline-5-sulfonyl chloride[16] with ethanol. The condensation with pyridine-2,6-dicarbaldehyde under acidic conditions led to the simultaneous hydrolysis of the ester and to the formation of the expected sulfonic acid **3k**.

The synthesis of compounds **7a**–**b** from the appropriate isatine **4** and 1,4-diacetylpiperazine-2,5-dione **6** has been performed in dimethylformamide in the presence of triethylamine.[17] In this reaction the condensation took place with contemporaneous hydrolysis of the acetyl group. When the indolaldehyde **5** was employed in place of the isatine **4** for the synthesis of compounds **8a**–**b**, this procedure gave poor yields whereas a better result was obtained with acetic anhydride/sodium acetate. Compounds **9a**–**b** were obtained by alkaline hydrolysis of the corresponding acetyl derivatives **8a**–**b**.

All compounds **3** were obtained as pure geometrical isomers and the configuration was assigned by means of ^{1}H-NMR spectra (Table S1). We performed Nuclear Overhauser Enhancement (NOE) analysis on compound **3n**: the spectrum shows that the geometrical configuration of the two possible centers is the same, since the protons at the positions 3 and 5 of the pyridine ring give a single signal. The same happens for the couple of NH groups and for the other couples of indole protons/substituents: H-4, H-7, OCH_3-5, CH_3-6. The first NOE analysis was devoted to the identification of H-4 and H-7 in the indolinone system. The irradiation of the NH groups (10.39 ppm) and of the 6-methyl groups (2.04 ppm) produced NOE at 6.62 ppm (peak which was assigned to H-7) whereas the irradiation of the 5-methoxy groups (3.33 ppm) produced NOE at 8.01 ppm (H-4). Therefore the remaining singlet of the aromatic region (7.67 ppm) was assigned to the methine groups. When this peak was irradiated, NOE was observed only at the protons in position 3 and 5 of the pyridine ring (7.90 ppm) therefore compound **3n** belongs to the E configuration.

Also compounds **7**–**9** were obtained as pure isomers and for the study of the geometrical configuration of these derivatives, NOE analyses were performed on compound **8a**. As observed in the previous case, the ^{1}H-NMR spectrum shows a symmetrical molecule i.e. the two centers have the same configuration. The irradiation of the NH group (10.44 ppm) gave NOE at the indole protons in position 2 (8.39 ppm) thus allowing us to confirm that the other singlet in the aromatic region (6.99 ppm) was due to the methine bridges. When these protons were irradiated, NOE was observed only at 7.74 ppm (which is a doublet of the indole protons at the 4 position: as a consequence the doublet at 8.38 ppm was attributed to the indole protons at the 7-position). This is in agreement with the Z configuration. The irradiation of the CH_3CO groups (2.77 ppm) produced NOE only at the indole protons in position 2 (8.39 ppm) and not at the methine bridges, thus confirming the Z configuration.

Antitumor activity

As a primary screening, compounds **3** and **7**–**9** were submitted to the Developmental Therapeutics Program (DTP) at the National Cancer Institute (NCI) (http://dtp.nci.nih.gov) for evaluation of antitumor activity in the human cell line screen. In a preliminary screen at a single concentration (100 μM) against three human cell lines (NCI-H460 lung cancer, MCF7 breast cancer and SF-268 glioma) a compound is considered active when it reduces the growth of any of the cell lines to 32% or less. Most of the compounds reported in Scheme 1 were active and passed on for evaluation in the full panel of sixty human tumor cell lines. This panel is organized into subpanels representing leukemia, melanoma and cancers of lung, colon, kidney, ovary, breast, prostate and central nervous system.

The test compounds were dissolved in DMSO and evaluated using five concentrations at ten-fold dilutions, the highest being 10^{-4} M. Table 2 reports the results obtained, expressed as the negative log of the molar concentration at three assay endpoints: the 50% growth inhibitory power (pGI_{50}), the cytostatic effect (pTGI=Total Growth Inhibition) and the cytotoxic effect (pLC_{50}). Some of the compounds were reassayed based on the results of the first screen: compounds **3d**, **3h**, and **3n** were assayed twice, compounds **3b** and **3i** were assayed three times and all other compounds were assayed once. The pyridine derivatives (**3**) were far more active than the piperazine derivatives (**7**–**9**). Compounds **3a**–**d**, **g**, **h**, **j**, **l**, **o** and **p** were potent growth inhibitors with mean pGI_{50} ranging from 5.08 to 6.34 and cytotoxicity from 4.04 to 4.85. The difference between the average concentration which caused 50% growth inhibition and the concentration which killed 50% of the cells was around 1.2 log with only 0.78 for **3o** and 1.71 for **3b**. Within each of the nine cell panels there was a difference of 0.9 between the least-sensitive and the most-sensitive (leukemia) cell lines in response to **3b**.

A comparison of the activities of the compounds reported in this paper shows that the weak activity of **7**–**9** is not related to the methoxy group in the indole system, to the acetyl group in the piperazine ring or to the methine bridge. Even with this limited number of compounds, it seems clear enough that the core is important since in the pyridyl derivatives **3a-q** the antitumor activity is evident and related to the substituents in the indole system. The presence of acidic groups led to loss of activity probably because the resulting compounds (**3f**, **i**, **k**) are too hydrophilic (in particular compounds **3f**, **i** showed their highest activity on leukemia cells). This hypothesis is confirmed by the weak activity of the hydroxy derivatives **3e**, **m**, **q**. Interesting results were obtained with halogens, methoxy and dimethylamino groups. The compound containing chlorine at the 4 position of the indole (**3b**) was very active: the activity decreased by shifting the halogen from the 4 to the 5 (**3c**) and 6 position (**3l**).

COMPARE analyses

We used the COMPARE tool from the DTP at the NCI to analyze the screening results of the most active compounds for indications of mechanism of action. COMPARE uses the pattern

of relative sensitivity/insensitivity of the cell lines in the screen to identify other compounds with similar patterns of activity or to identify molecular targets whose patterns of expression correlate with the pattern of compound activity.

Matrix COMPARE was used to determine the pair-wise correlation of the cell response pattern for every combination of two compounds in the set of nine compounds for which we had data at the GI_{50} endpoint. In addition, the individual compound screening results at the GI_{50} endpoint were also submitted for COMPARE analyses against the NCI standard agent compound set, against all of the NCI public compound screening data and against all of the NCI public microarray expression data. COMPARE correlations of 0.6 or greater between two compounds suggest that the compounds affect the same mechanism(s) within cells.[18]

In the matrix COMPARE analysis of the nine compounds, the highest correlation was 0.74 between **3c** and **3h**, and the second highest correlation of 0.65 was between **3c** to **3o**. The direct correlation between **3h** and **3o** was 0.48. Qualitative inspection of the relative sensitivities in the cell line panels indicates that these correlations are driven by greater-than-average activity against leukemia and melanoma cell lines coupled with less-than-average activity against non-small cell lung and renal cell lines. Within the matrix COMPARE results, correlations other than those above had an average of about 0.19 with a standard deviation of 0.19, and were not considered further.

COMPARE has not identified any single strong candidates for mechanism of action or molecular targets that may be targeted by the bis-indole derivatives, though the correlations among a few compounds in this series, along with high correlations to some related compounds previously submitted, suggest commonalities among them. It may be that the bis-indole derivatives are affecting several different cellular processes at the same time producing cell response patterns which do not correlate with any public compounds previously submitted to COMPARE. It is also possible that these compounds do not satisfy the important assumptions when using COMPARE to search for a mechanism of action: compounds with similar mechanism(s) or the relevant molecular target(s) must already have been assayed and included in the database, and the standard 48-hour exposure time in the screening assay must have been sufficient for a compound's effects to manifest themselves; and, in addition, the cell lines in the screen must have exhibited a range of sensitivities to the compounds or a range of expression of the molecular targets so that there are useful patterns for COMPARE to work with.

Further studies for compounds 3b and 3d

Compounds **3b** and **3d**, with pGI_{50} values of 6.34 and 5.92 respectively, were among the most active of the whole series. In a collaboration with Oncotest (Freiburg, Germany) these compounds were subjected to a further *in vitro* evaluation on a panel of 36 human tumor cell lines (bladder, colon, CNS, gastric, head-neck, lung, breast, melanoma, ovarian, pancreas, prostate, pleuramesothelioma, renal, uterus body) and the result was subjected to COMPARE analysis against the company's own database of compound screening data. This is a similar methodology to the NCI COMPARE above, but used a separate set of screening data obtained from a distinct set of cell lines.

Compound **3b** was the closest match with **3d**. Overall, **3b** was more potent than **3d** (mean IC_{70} 0.35 versus 0.56 μg/ml) and has a more pronounced selectivity. Compounds **3b** and **3d** were similar in the COMPARE analyses (r=0.60) and **3b** was correlated with Tyropeptin A, a proteasome inhibitor, with a correlation of r=0.54. This result prompted us to test whether compound **3b** was a proteasome inhibitor. The study was performed at the University of Maryland after taking into account the high growth inhibitory effect of compound **3b** against breast cancer cells and its comparable in vitro growth inhibitory activity to the known proteasome inhibitor MG-132 (Z-Leu-Leu-Leu-al) as shown in Figure 1. The ubiquitin-

proteasome system regulates cellular protein degradation and homeostasis. In fact, many of the known E3 ligases that tag a given protein with ubiquitin targeting it for degradation in the proteasome play a role in various diseases, including cancer development and progression. In particular, breast cancer seems to be associated with the aberrant expression of several RING E3 ligases including BCA2.[19] E3 ligases are very unstable because of autoubiquitination, a hallmark of E3 activity, and their rapid degradation in the proteasome.[19] Although, compound **3b** and MG-132 had very similar activity in the considered breast cancer cell line panel (Figure 1 and Table 3), their behavior was very different when we examined the compounds ability to influence the stability of endogenous BCA2, via inhibition of the proteasome. While MG-132 can stabilize the protein, in the presence of the protein synthesis inhibitor cycloheximide (CHX), **3b** had only very limited effects. However some potential to stabilize BCA2 was seen at 16 h (lanes 3 and 5, Figure 2) in that **3b** treatment led to an approximately 2-fold increase of BCA2 protein compared to CHX treatment. However, MG-132 was able to stabilize BCA2 more than 10-fold compared to the CHX treatment (Figure 2). In particular at 8 h a second, smaller band appeared in the Western blot. This band is likely a form of BCA2 that is phosphorylated by AKT as shown before, suggesting that **3b** might modulate the stability of kinases such as AKT.[20]

No effects of **3b** were found on β-actin (used as loading control) or the oncogenic receptor C-erbB2, a protein that is degraded via the lysosomal pathway (Figure 2).

PMET inhibition

Another potential mechanism of action of anticancer agents is the inhibition of plasma membrane electron transport (PMET), a stress adaptation pathway that has been associated with tumor activity.[21,22] Since these compounds could exhibit a potential redox activity due to the presence of ketone groups, we thought it was useful to determine whether the *in vitro* activity was associated with the ability to inhibit PMET. Therefore compounds active against HL60 cells and some inactive compounds were selected (**3b**–**e**, **g**–**i**, **l**–**o**, **8a**,**9a**) and tested at the Malaghan Institute of Medical Research (New Zealand) in human leukemic HL60 cells and mitochondrial gene-knockout HL60ρ^0 cells which have defective mitochondrial electron transport and therefore exclude possible respiratory effects. PMET was assayed by measuring the extracellular reduction of the tetrazolium dye, WST-1, in the presence of the intermediate electron acceptor, 1-methoxyphenazinemethosulfate (mPMS). Compounds **3g**, **3i**, **3o**, **8a** and **9a** were inactive at 200 μM while IC_{50}s for the other compounds were 117 μM or greater in both cell lines. In contrast, the IC_{50} values for cell proliferation as determined in a 2-day MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] cell proliferation assay for compounds **3b** and **3c** were 3–8 μM for both HL60 and HL60 ρ^0 cells. Other experiments, performed at the Biochemistry Department - University of Bologna, showed that inhibition of PMET occurred in different human leukemic cell lines, such as B1647, HEL and M07e, only in the presence of **3b** (IC_{50} 15–30 μM) (Figure 3), **3d** (IC_{50} 30–45 μM) and, to a lesser extent, **3e** (IC_{50} 40–50 μM). These data correlate with effects on mitochondrial Complex I and on total respiratory chain activity. In these cell lines the MTT assay confirmed the antiproliferative activity of all tested compounds. Results here reported underline that PMET inhibitory activity of compounds **3b**, **3d** and **3e** may contribute to their antiproliferative effect in some leukemic cell lines but, in general, PMET inhibitory activity does not correlate closely with antiproliferative effect in the leukemic cells tested.

In vivo experiments

Further studies continued at the NCI where the maximum tolerated dose (MTD) was determined for compounds **3b** and **3d** and it was found high enough (400 mg/Kg) to warrant further testing which started with **3d** only. The first *in vivo* experiment was the "hollow fiber

assay".[23] Compound **3d** was tested against a panel of six tumor cell lines consisting of the leukemia cell lines HL-60, K562, MOLT-4, CCRF-CEM, RPMI-8226, SR. A compound is considered for xenograft testing if it has a combined i.p. + s.c. score of 10 or greater, a s.c. score of 4 or greater, or produces cell kill of any cell line at either dose level evaluated. Compound **3d** resulted in a i.p. + s.c. score of 22 and it was therefore examined for distal site antitumor activity in an appropriate human tumor xenograft model in nude mice[24,25] (Table S2). As shown in Table S3 it did not show significant activity in the tumor employed (HL-60-TB leukemia).

In conclusion we demonstrated that this new approach was successful since some of the compounds here described are much more active than the numerous, so far prepared and tested 3-indolylmethylene-2-indolinones which have been mentioned in the introduction as the rationale for this search.

Experimental section

A) Chemistry

The melting points are uncorrected. Elemental analyses were performed with a Fisons Carlo Erba Instrument EA1108 and resulted within 0.4% of the theoretical values (Table S4). Bakerflex plates (silica gel IB2-F) were used for TLC: the eluent was petroleum ether/acetone in various proportions. The IR spectra were recorded in nujol on a Nicolet Avatar 320 E.S.P.; ν_{max} is expressed in cm^{-1}. The ^{1}H-NMR spectra were recorded in $(CD_3)_2SO$ or CF_3COOD on a Varian Gemini (300 MHz); the chemical shift (referenced to solvent signal) is expressed in δ (ppm) and J in Hz. For the spectra which are not reported here see Table S1.

Pyridine-2,6-dicarbaldehyde and all the starting indolinones **1**, except ethyl 2-oxoindoline-5-sulfonate, are commercially available or have been prepared as described in the literature (see Table 1).[9,12,13,26–33] Compounds **7**–**9** were prepared from commercially available starting material.

Synthesis of ethyl 2-oxoindoline-5-sulfonate—2-Oxoindoline-5-sulfonyl chloride (6 mmol) was refluxed for 30 min. with 30 ml of ethanol. The white crystalline precipitate formed after cooling was separated by filtration with a yield of 90% and used without further purification.

$C_{10}H_{11}NO_4S$ Mw 241.27 mp 150°C. IR: 1716, 1614, 1004, 922, 784. NMR: 1.19 (3H, t, CH_3-CH_2, J=7.2) 3.61 (2H, s, ind-3), 4.04 (2H, q, CH_3-CH_2), 7.01 (1H, d, ind-7, J=8), 7.69 (1H, s, ind-4), 7.73 (1H, d, ind-6, J=8), 10.90 (1H, s, NH).

Synthesis of 3,3'-[pyridine-2,6-diylbis(methylene)]bis(1,3-dihydroindol-2-ones) (3a–q)—Two different methods were employed according to the substituents in the indole system (Table 1).

Method 1 (compounds **3e**, **g**, **h**, **l**-**n**, **p**, **q**). This method was emplyed even for the published compounds **3a**–**d**, **o**.[12,13] The appropriate indolinone **1** (10 mmol) was dissolved in methanol and treated with pyridine-2,6-dicarbaldehyde **2** (5 mmol) and piperidine (2 ml). The reaction mixture was refluxed for 3–5 h (according to a TLC test), cooled and, if necessary, concentrated at reduced pressure. The yellow to orange precipitate thus formed was collected by filtration with a yield of 80% for compounds **3n** and 50–60% for the others. They were used as such without further purification.

Data for **3g**. IR: 3283, 1710, 1677, 1214, 1091. ^{1}H-NMR: 2.52 (12H, s, CH_3), 6.60 (2H, dd, ind-6, J=8.4 Hz, J=2.2 Hz), 6.66 (2H, d, ind-7, J=8.4 Hz), 7.67 (2H, s, -CH=), 7.87(2H, d,

ind-4, J=2.2 Hz), 7.89 (2H, d, py-3,5, J=7.8 Hz), 8.09 (1H, t, py-4, J=7.8 Hz), 10.28 (2H, s, NH). Anal. $C_{27}H_{25}N_5O_2$ (C, H, N).

Data for **3h**. IR: 3100, 1695, 1617, 1194, 912. ^{1}H-NMR: 6.76 (2H, m, ind), 6.91 (2H, m, ind), 7.81 (2H, s, -CH=), 7.98 (4H, m, ind+py-3,5), 8.15 (1H, t, py-4, J=7 Hz), 10.65 (2H, s, NH). Anal. $C_{23}H_{13}F_2N_3O_2$ (C, H, N).

Data for **3l**. IR: 3201, 1721, 1611, 1070, 917. ^{1}H-NMR: 6.48 (2H, dd, ind-5, J=8.1 Hz, J=1.5 Hz), 6.81 (2H, d, ind-7, J=1.5 Hz), 7.44 (2H, s, -CH=), 7.94 (2H, d, py-3,5, J=7.8 Hz), 8.11 (1H, t, py-4, J=7.8 Hz), 8.12 (2H. d, ind-4, J=8.1 Hz), 10.78 (2H, s, NH). Anal. $C_{23}H_{13}Cl_2N_3O_2$ (C, H, N).

Method 2 (compounds **3f**, **i**–**k**). Pyridine-2,6-dicarbaldehyde **2** (10 mmol) was dissolved in acetic acid (50 ml) and treated with the appropriate indolinone **1** (20 mmol) and 37% hydrochloric acid (1 ml). The reaction mixture was refluxed for 3–5 h (according to a TLC test) and cooled. The yellow to orange precipitate thus formed was collected by filtration with a yield of 80% for **3k** and 50–60% for the others. They were used as such without further purification.

Data for **3j**. IR: 3170, 1706, 1609,1301, 717. ^{1}H-NMR: 6.86 (2H, d, ind-7, J=8.5 Hz), 7.49 (2H, dd, ind-6, J=8.5 Hz, J=1.2 Hz), 8.01 (2H, s, -CH=), 8.07 (2H, d, ind-4, J= 1.2 Hz), 8.27 (1H, t, py-4, J=7.5 Hz), 8.65 (2H, d, py-3+5, J=7.5 Hz), 11.11 (2H, s, NH). Anal. $C_{23}H_{13}Br_2N_3O_2$ (C, H, N).

Synthesis of 3,6-bis(2-oxo-1,2-dihydroindol-3-ylidene)piperazine-2,5-diones (7a–b)—The appropriate isatine (**4a**–**b**, 5 mmol) was added to a solution of 1,4-diacetylpiperazine-2,5-dione **6** (2.5 mmol) in DMF (10 ml). After the addition of triethylamine (5.5 mmol), the reaction mixture was stirred at room temperature for 3–5 h. The brown precipitate thus formed was collected by filtration with a yield of 90% and used without further purification.

Synthesis of 1,4-diacetyl-3,6-bis(indol-3-ylmethylene)piperazine-2,5-diones (8a–b)—The appropriate indolealdehyde **5** (10 mmol) was treated with 1,4-diacetylpiperazine-2,5-dione **6** (5 mmol), anhydrous sodium acetate (20 mmol) and acetic anhydride (10 ml). The reaction mixture was refluxed for 3–5 h (according to a TLC test) and acetic anhydride was removed under reduced pressure. The residue was treated with ice water and the resulting yellow to orange precipitate was collected by filtration with a yield of 70% and used without further purification.

Synthesis of 3,6-bis(indol-3-ylmethylene)piperazine-2,5-diones (9a–b)—The appropriate compound **8** (1 mmol) was dissolved in ethanol (250 ml) and refluxed for 5 h with 2N KOH (3 ml). The yellow to orange precipitate formed after cooling was collected by filtration and used without further purification. The yield was 60% for **9a** and 30% for **9b**.

B) Biology

a) *In Vitro* Growth Inhibition and Cytotoxicity—It was determined on the 3 and on the 60 cell line panel by the NCI according to standard procedures.[34]

b) 36 Cell line assay—The 36 cell line assay was performed at Oncotest: 24 of the 36 cell lines were established from patient-derived tumor xenografts passaged subcutaneously in nude mice.[35] The origin of the donor xenografts has been already described.[36,37] The other 12 cell lines were commercially available. All cells were grown at 37°C in a humidified atmosphere (95% air, 5% CO_2) in RPMI 1640 medium (PAA, Cölbe, Germany) supplemented with 10%

fetal calf serum (PAA) and 0.1 mg/ml gentamicin (PAA). A modified propidium iodide assay[38] was used to assess the effects of compounds. Tumor derived cell lines were incubated in 96 multi-well plates. After one day, the compounds under test were added to the plates at 5 concentrations in the range from 0.001 mg up to 10 mg and left for further four days. The inhibition of proliferation was determined by measuring the DNA content using an aqueous propidium iodide solution (7 mg/ml). Fluorescence was measured using the Cytofluor 4000. All compounds were tested in three independent experiments. In each experiment, all data points were determined in triplicate.

The COMPARE Algorithm uses *in vitro* activity data to obtain clues as to the mechanism of action of a test compound. The individual IC_{70}-values of the test compounds in 36 test cell lines obtained in the monolayer assay were correlated to the corresponding IC_{70}-values for more than 100 standard agents determined in exactly these 36 cell lines. Data for these standard agents are available in the Oncotest database.

c) Analysis of BCA2 protein stability—To determine the stability of endogenous BCA2, 1×10^6 MCF7 cells were seeded into six-well plates and grown overnight. The protein synthesis inhibitor cyclohexamide (Sigma) was added alone (100 μmol/L) or in combination with the proteasome inhibitor MG-132 (10 μmol/L, Calbiochem) or compound **3b** for a total of 8 and 16 hours. Cell lysates were generated and Western blots performed as described before.[39] We used the polyclonal anti-BCA2 antibody (dil. 1:800) that has been generated,[19] the β-actin monoclonal mouse antibody was from Sigma (St. Louis, MO, dil. 1:5000) and the monoclonal anti-C-erbB2 antibody (dil. 1:2000) from EMD Bioscienes, La Jolla, CA.

Signals were quantified using the NIH ImageJ software by measuring the mean signal intensity (grey scale) value and its integrated density. Resulting absolute intensities were determined and a relative intensity value obtained by dividing the absolute intensity by the absolute intensity of the control sample. MTT cytotoxicity assays were performed with the breast cancer cell lines in Table 3 as described before.[39]

d) PMET and MTT cell proliferation assays—Inhibition of PMET was determined essentially as described [22] using a microplate format with 100,000 cells per well in 0.1 ml. Cells were pretreated for 30 min with compound dissolved in DMSO (<0.1% final concentration) prior to adding WST-1/mPMS and WST-1 reduction measured in real time at 450 nm in a BMG Fluostar microplate reader. MTT reduction was measured following incubation of cells with compound for 48 h.

e) MTD assay (http://dtp.nci.nih.gov)—Basically what is done is to dose a mouse with a single injection of 400 mg/kg, i.p.. If the animal survives 14 days, the MTD is 400. If the animal dies, the dose is lowered to 200 mg/kg. The dose keeps being lowered by 50% until a tolerated dosage is determined and that will be the MTD.

f) Hollow fiber assay and xenograft—These tests were performed by the NCI according to standard procedures (http://dtp.nci.nih.gov/branches/btb/hfa.html).[24,25]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations

NOE, Nuclear Overhauser Enhancement
DTP, Developmental Therapeutics Program

NCI, National Cancer Institute
GI, Growth Inhibition
TGI, Total Growth Inhibition
LC, Lethal Concentration
DAVID, Database for Annotation, Visualization and Integrated Discovery
PMET, plasma membrane electron transport
mPMS, 1-methoxyphenazinemethosulfate
WST-1, water soluble tetrazolium salt-1
MTT 3-(4,5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide
CHX, cycloheximide
RSI, relative signal intensity
MTD, maximum tolerated dose

Acknowledgements

This work has been supported by a grant from MIUR-COFIN 2006. We are grateful to the National Cancer Institute (Bethesda, MD) for the anticancer tests and to Professor Romana Fato for the studies on mitochondrial Complex I and on total respiratory chain.

References

1. Potential Antitumor Agents. 43 (hawks-1). For part 42, seeAndreani A, Burnelli S, Granaiola M, Leoni A, Locatelli A, Morigi R, Rambaldi M, Varoli L, Calonghi N, Cappadone C, Farruggia G, Zini M, Stefanelli C, Masotti L, Radin NS, Shoemaker RH. New Antitumor Imidazo[2,1-*b*]thiazole Guanylhydrazones and Analogues. J. Med. Chem. In press
2. Andreani A, Rambaldi M, Bonazzi D, Lelli G, Bossa R, Galatulas I. Substituted Imidazo[2,1-b] thiazoles and Thiazolines as Potential Cardiotonic Agents. Eur. J. Med. Chem 1984;19:219–222.
3. Andreani A, Rambaldi M, Locatelli A, Bossa R, Galatulas I, Ninci M. Synthesis and Cardiotonic Activity of 2-Indolinones. Eur. J. Med. Chem 1990;25:187–190.
4. Andreani A, Locatelli A, Rambaldi M, Leoni A, Bossa R, Fraccari A, Galatulas I. Potential Antitumor Agents. 25. Synthesis and Cytotoxic Activity of 3-(2-Chloro-3-indolylmethylene)1,3-dihydroindol-2-ones. Anticancer Res 1996;16:3585–3588. [PubMed: 9042225]
5. von Dobeneck H, Wolkenstein D, Blankenstein G. α,β'-Diindolylmethane und – methene Der Urorosein-Chromophor. Chemische Berichte 1969;102:1347–1356.
6. Kobayashi G, Matsuda Y, Tominaga Y, Ohkuma M, Shinoda H, Kohno M, Mizuno D. Antitumor Activity of Indole Derivatives. Yakugaku Zasshi 1977;97:1033–1039. [PubMed: 925872]
7. Buzzetti F, Pinciroli V, Brasca MG, Crugnola A, Fustinoni S, Longo A. Synthesis and Configuration of Some New Bicyclic 3-Arylidene-and 3-Heteroarylidene-2-oxindoles. Gazz. Chim. Ital 1995;125:69–75.
8. Andreani A, Locatelli A, Leoni A, Morigi R, Chiericozzi M, Fraccari A, Galatulas I, Salvatore G. Synthesis and Potential Coanthracyclinic Activity of Pyridylmethylene and Indolylmethylene Lactams. Eur. J. Med. Chem 1998;33:905–909.
9. Andreani A, Granaiola M, Leoni A, Locatelli A, Morigi R, Rambaldi M, Garaliene V. Synthesis and Antitumor Activity of 1,5,6-Substituted 3-(2-chloro-3-indolylmethylene)1,3-dihydroindol-2-ones. J. Med. Chem 2002;45:2666–2669. [PubMed: 12036377]
10. Andreani A, Granaiola M, Leoni A, Locatelli A, Morigi R, Rambaldi M, Garaliene V, Farruggia G, Masotti L. Substituted *E*-3-(2-Chloro-3-indolylmethylene)1,3-dihydroindol-2-ones With Antitumor Activity. Bioorg. Med. Chem 2004;12:1121–1128. [PubMed: 14980624]
11. Andreani A, Burnelli S, Granaiola M, Leoni A, Locatelli A, Morigi R, Rambaldi M, Varoli L, Calonghi N, Cappadone C, Farruggia G, Zini M, Stefanelli C, Masotti L. Substituted *E*-3-(2-Chloro-3-indolylmethylene)1,3-dihydroindol-2-ones with Antitumor Activity. Effect on the Cell Cycle and Apoptosis. J. Med. Chem 2007;50:3167–3172. [PubMed: 17559205]

12. Andreani A, Rambaldi M, Leoni A, Locatelli A, Bossa R, Chiericozzi M, Dambrosio M, Galatulas I. Synthesis and Cardiotonic Activity of 2-Indolinones Bearing Pyridyl groups. Eur. J. Med. Chem 1993;28:653–657.

13. Andreani A, Leoni A, Locatelli A, Morigi R, Chiericozzi M, Fraccari A, Galatulas I. Potential Coanthracyclinic Activity of Pyridylmethylene-2-indolinones. Anticancer Res 1998;18:3407–3410. [PubMed: 9858916]

14. Tanaka K, Konno Y, Kuraishi Y, Kimura I, Suzuki T, Kiniwa M. Synthesis of a Magnosalin Derivative, 4-(3,4,5-Trimethoxyphenyl)-6-(2,4,5-trimethoxyphenyl)-2-diethylaminopyrimidine, and the Anti-Angiogenic and Anti-Rheumatic Effect on Mice by Oral Administration. Bioorg. Med. Chem. Lett 2002;12:623–627. [PubMed: 11844686]

15. Doggrell SA. RITA-A Small-molecule Anticancer Drug that Targets p53. Expert Opin. Investig. Drugs 2005;14:739–742.

16. Barlaam B, Bird TG, Lambert-van der Brempt C, Campbell D, Foster SJ, Maciewicz R. New r-Substituted Succinate-Based Hydroxamic Acids as TNFr Convertasi Inhibitors. J. Med. Chem 1999;42:4890–4908. [PubMed: 10579851]

17. Katritzky AR, Fan W-Q, Szajda M, Li Q-L, Caster KC. Conjugated Systems Derived from Piperazine-2,5-dione. J. Heterocyclic Chem 1988;25:591–597.

18. Paull KD, Lin CM, Malspeis L, Hammel E. Identification of Novel Antimitotic Agents Acting at the Tubulin Level by Computer-assisted Evaluation of Differential Cytotoxicity Data. Cancer Res 1992;52:3892–3900. [PubMed: 1617665]

19. Burger A, Amemiya Y, Kitching R, Seth AK. Novel RING E3 Ubiquitin Ligases in Breast Cancer. Neoplasia 2006;8:689–695. [PubMed: 16925951]

20. Connor MK, Azmi PB, Subramaniam V, Li H, Seth A. Molecular Characterization of Ring Finger Protein 11. Molecular Cancer Research 2005;3:453–461. [PubMed: 16123141]

21. Herst PM, Berridge MV. Plasma Membrane Electron Transport: a New Target for Cancer Drug Development. Curr. Mol. Med 2006;6:895–904. [PubMed: 17168740]

22. Herst PM, Petersen T, Jerram P, Baty J, Berridge MV. The Antiproliferative Effects of Phenoxodiol are Associated with Inhibition of Plasma Membrane Electron Transport in Tumour Cell Lines and Primary Immune Cells. Biochem. Pharm 2007;74:1587–1595. [PubMed: 17904534]

23. Hollingshead M, Alley MC, Camalier RF, Abbott BJ, Mayo JG, Malspeis L, Grever MR. *In Vivo* Cultivation of Tumor Cells in Hollow Fibers. Life Sciences. 1995;57:131–141.

24. Alley, MC.; Hollingshead, MG.; Dykes, DJ.; Waud, WR. Human Tumor Xenograft Models in NCI Drug Development. In: Teicher, BA.; Andrews, PA., editors. Cancer Drug Discovery and Development: Anticancer Drug Development Guide: Preclinical Screening, Clinical Trials, and Approval. Vol. 2nd Ed.. Totowa, NJ: Humana Press Inc; 2004.

25. Plowman, J.; Dykes, DJ.; Hollingshead, M.; Simpson-Herren, L.; Alley, MC. Human Tumor Xenograft Models in NCI Drug Development. In: Teicher, B., editor. Anticancer Drug Development Guide: Preclinical Screening, Clinical Trials, and Approval. Totowa, NJ: Humana Press Inc; 1997.

26. Beer RJS, Davenport HF, Robertson A. Extensions of the Synthesis of Hydroxyindoles from p-Benzoquinones. J. Chem. Soc 1953:1262–1264.

27. Nakagawa K, Sato T, Nishi T, Oshiro Y, Yamamoto K. Carbostyril and Oxindole Derivatives. Jpn. Kokai Tokkyo Koho. 1977:5.CODEN: JKXXAF JP 52073866 19770621 Showa. Patent written in Japanese. Application: JP 75-150935 19751216. CAN 87:167899 AN 1977:567899 CAPLUS

28. Minisci F, Galli R, Cecere M. Amminazione Radicalica di Composti Aromatici Attivati: Acetammidi. Nuovo Processo per la Sintesi di *para*-Ammino-N,N-dialchilaniline. La Chimica e l'Industria 1966;48:1324–1326.

29. Zakrzewska A, Kolehmainen E, Osmialowski B, Gawinecki R. 4-Fluoroanilines: Synthesis and Decomposition. J. Fluorine Chem 2001;111:1–10.

30. Hidenori O, Shigeharu T, Takafumi F, Shuji T, Kazumi K, Shuji Y, Youichi Y, Michiaki T, Kazuyuki N. Studies on Positive Inotropic Agents. Synthesis of 1-Heteroaroylpiperazine Derivatives. Chem. Pharm. Bull 1988;36:2253–2258. [PubMed: 3240459]

31. Sun L, Tran N, Tang F, App H, Hirth P, McMahon G, Tang C. Synthesis and Biological Evaluations of 3-Substituted Indolin-2-ones: A Novel Class of Tyrosine Kinase Inhibitors that Exhibit Selectivity

toward Particular Receptor Tyrosine Kinases. J. Med. Chem 1998;41:2588–2603. [PubMed: 9651163]

32. Andreani A, Rambaldi M, Bonazzi D, Greci L, Andreani F. Potential Antitumor Agents. III. Hydrazone Derivatives of 5-Substituted 2-Chloro-3-formyl-6-methylindole. Farmaco 1979;34:132–138.
33. Porter JC, Robinson R, Wyler M. Monothiophthalimide and Some Derivatives of Oxindole. J. Chem. Soc 1941:620–624.
34. Monks A, Scudiero D, Skehan P, Shoemaker R, Paull K, Vistica D, Hose C, Langley J, Cronise P, Vaigro-Wolff A. Feasibility of a High-flux Anticancer Drug Screen Using a Diverse Panel of Cultured Human Tumor Cell Lines. J. Natl. Cancer Inst 1991;83:757–766. [PubMed: 2041050]
35. Roth T, Burger AM, Dengler W, Willmann H, Fiebig HH. Fiebig HH, Burger AM. Human Tumor Cell Lines Demonstrating the Characteristics of Patient Tumors as Useful Models for Anticancer Drug Screening. In *Relevance of Tumor Models for Anticancer Drug Development*. Contrib. Oncol 1999;54:145–156.
36. Fiebig, HH.; Dengler, WA.; Roth, T. Human Tumor Xenografts: Predictivity, Characterization, and Discovery of New Anticancer Agents. In *Relevance of Tumor Models for Anticancer Drug Development*. In: Fiebig, HH.; Burger, AM., editors. Contrib. Oncol. Vol. 54. 1999. p. 29-50.
37. Fiebig, HH.; Berger, DP.; Dengler, WA.; Wallbrecher, E.; Winterhalter, BR. Combined *in vitro*/*in vivo* Test Procedure with Human Tumor Xenografts. In: Fiebig, HH.; Berger, DP., editors. Immunodeficient Mice in Oncology. Vol. 42. 1992. p. 321-351.Karger, Basel
38. Dengler WA, Schulte J, Berger DP, Mertelsmann R, Fiebig HH. Development of a Propidium Iodide Fluorescence Assay for Proliferation and Cytotoxicity Assay. Anti-Cancer Drugs 1995;6:522–532. [PubMed: 7579556]
39. Burger AM, Gao Y, Amemiya Y, Kahn HJ, Kitching R, Yang Y, Sun P, Narod SA, Hanna WM, Seth AK. A Novel RING-Type Ubiquitin Ligase Breast Cancer-Associated Gene 2 Correlates with Outcome in Invasive Breast Cancer. Cancer Res 2005;65:10401–10412. [PubMed: 16288031]

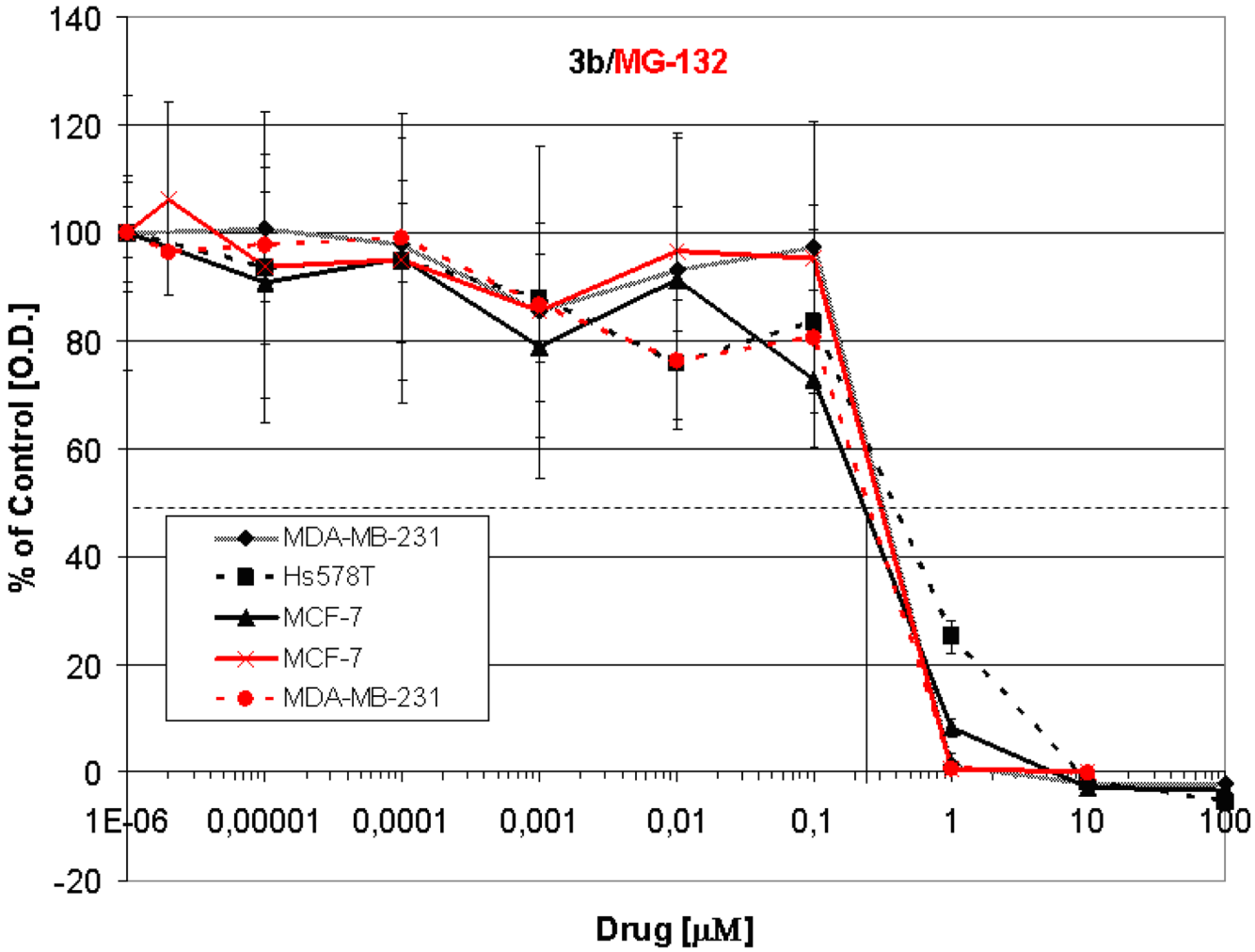


Figure 1. Antiproliferative activity of 3b (black lines) in a panel of breast cancer cell lines in comparison to the proteasome inhibitor MG-132 (red lines)

Growth curves are shown for effects of drugs after 5 days of continuous exposure. Proliferation was measured by MTT assay and data are displayed as percent growth inhibition compared to the growth of vehicle treated control cells.

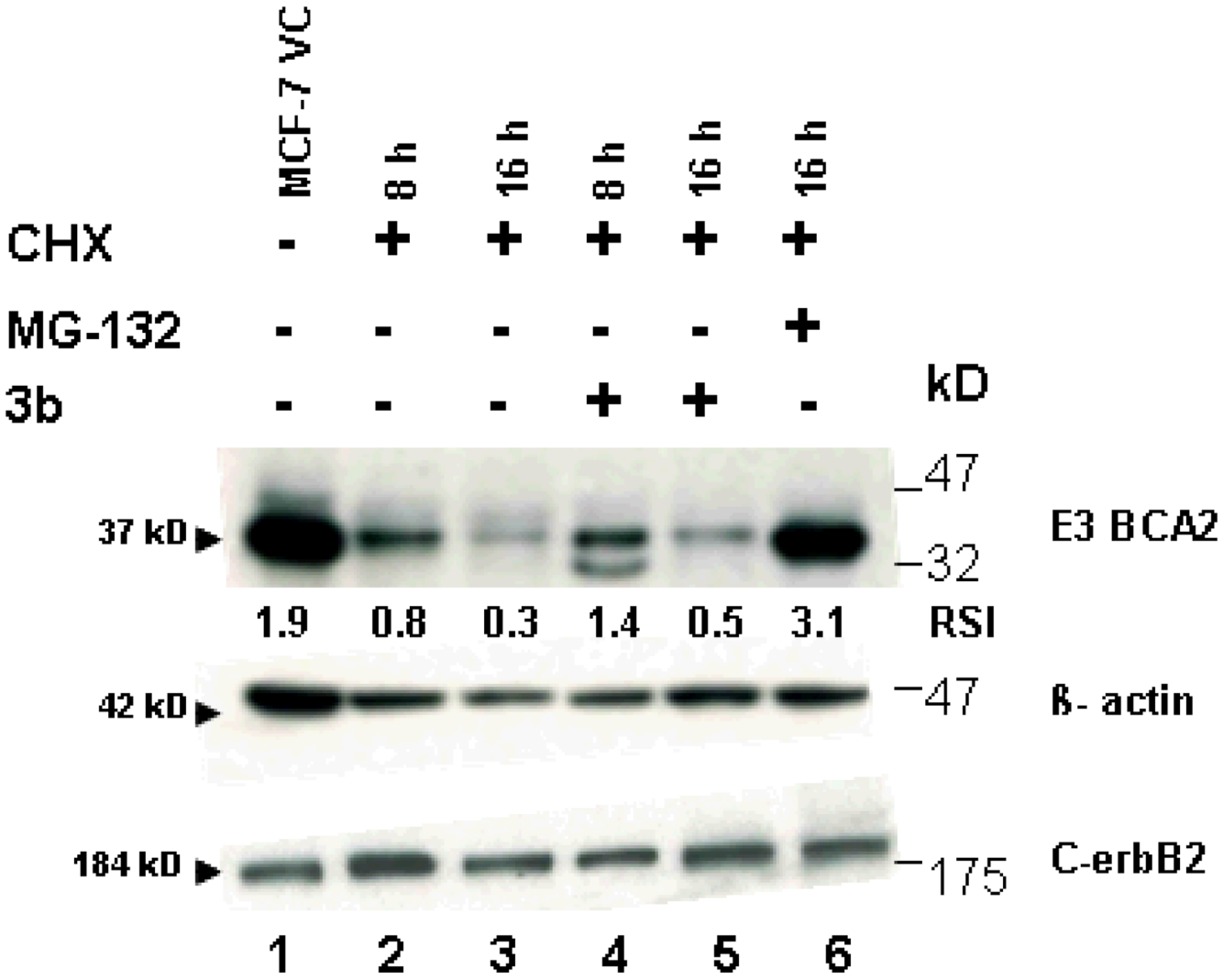


Figure 2. (VC, vehicle control)

Effects of **3b** on the stability of the ubiqutin E3 ligase BCA2 in MCF-7 breast cancer cells. The protein synthesis inhibitor CHX alone and in combination with the proteasome inhibitor MG-132 and **3b** were evaluated for effects on the protein stability of BCA2 (which is degraded in the proteasome), the stability of C-erbB2 (a receptor tyrosine kinase that is degraded in the lysosome) and the stable housekeeping gene β-actin. Expression levels of BCA2 relative to β-actin are shown below each lane of the BCA2 blot. Compound **3b** treated MCF-7 cells lysates exhibit approximately double the amount of BCA2 compared to CHX alone treated cells. The expression levels of C-erbB2 relative to β-actin ranged from 1.1 to 0.8 RSI (relative signal intensity) as determined by ImageJ software.

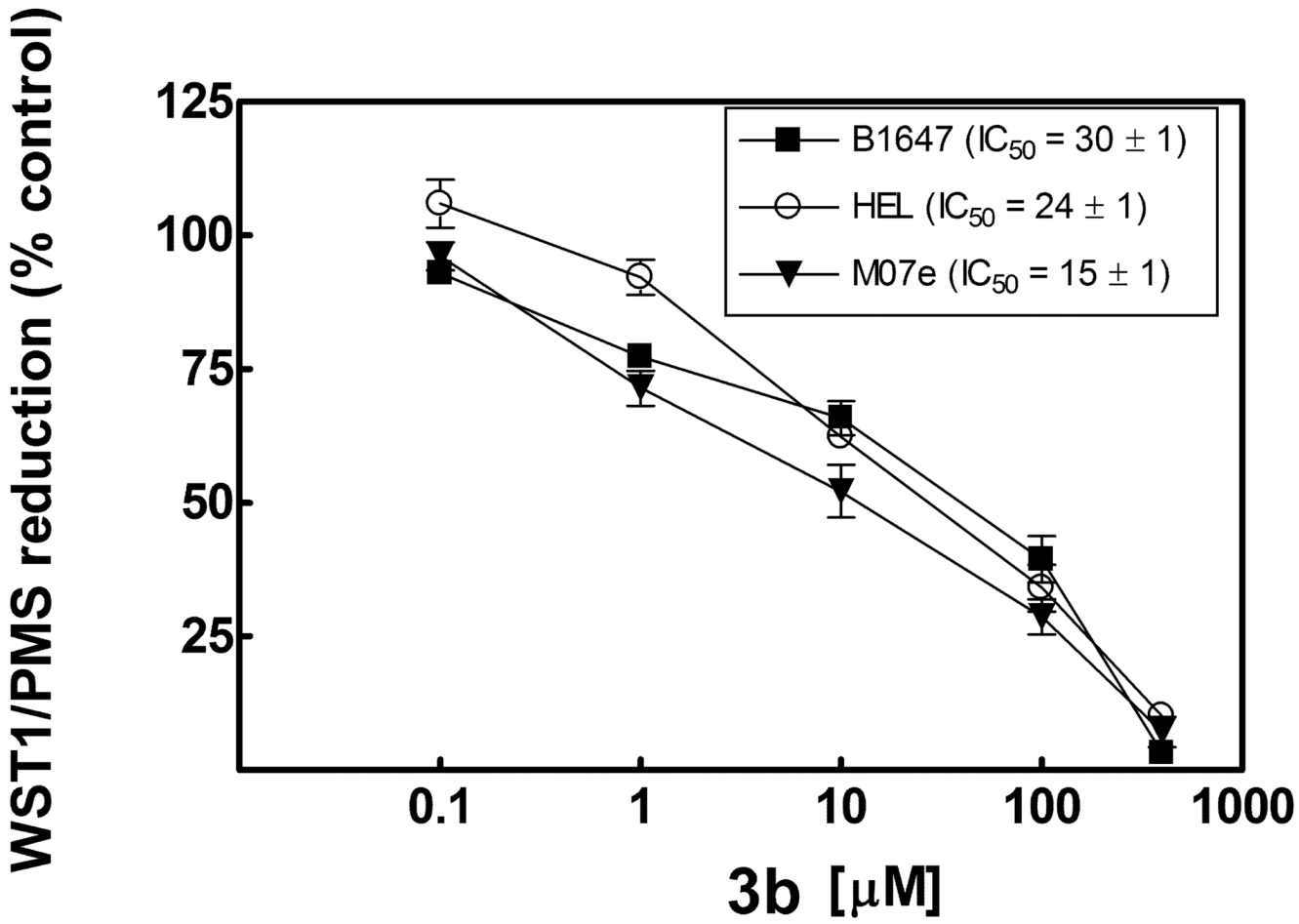
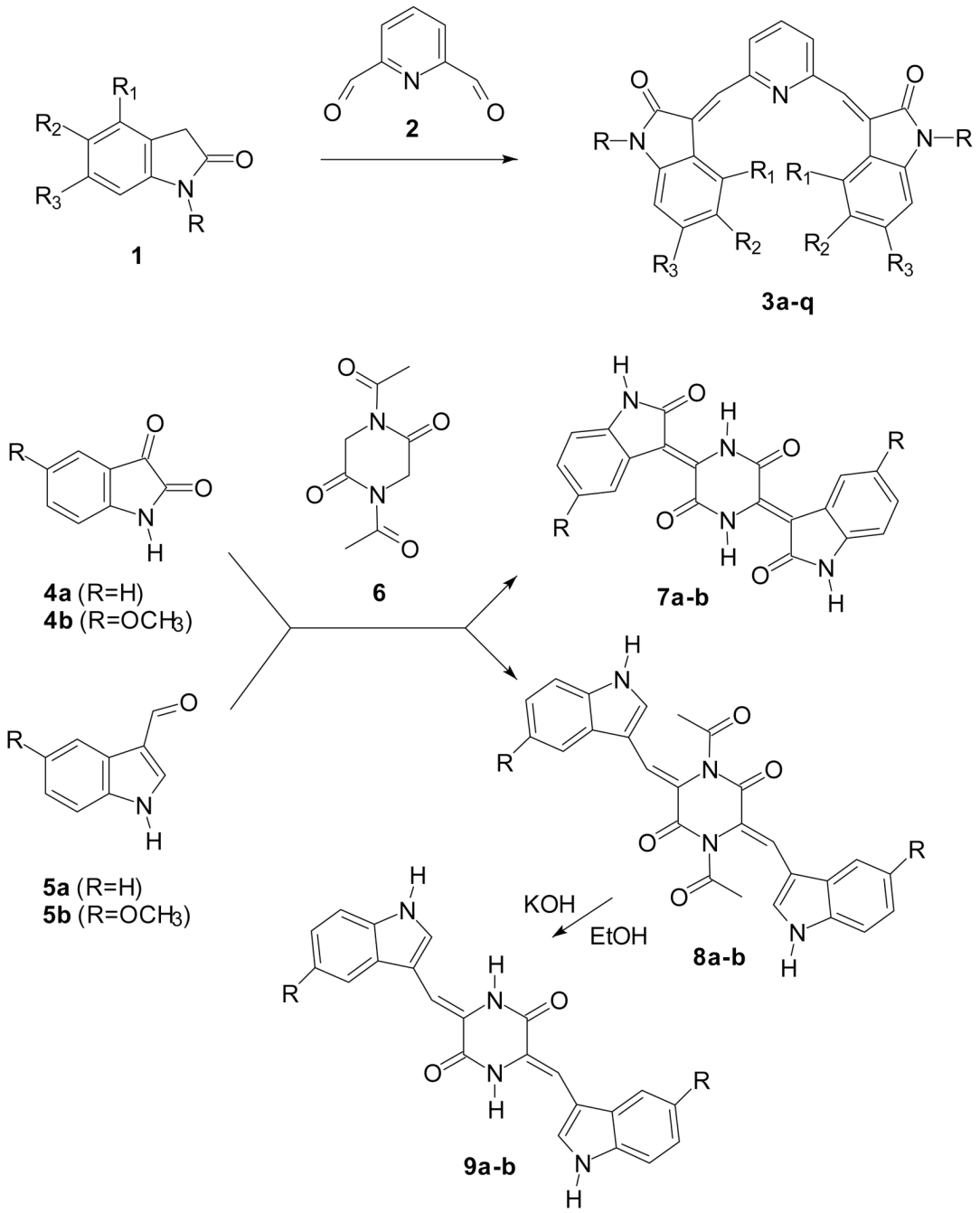


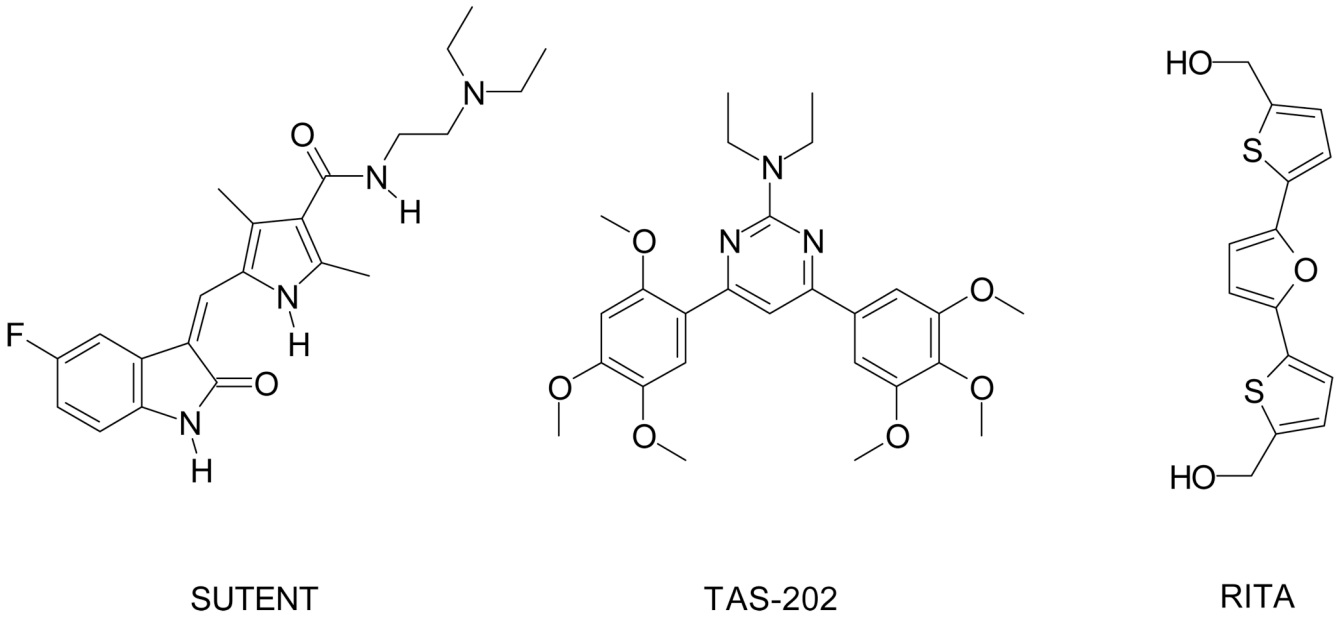
Figure 3. Effect of compound 3b on PMET activity in acute leukemic cell lines

Cells (10^5cells per well in 0.1 ml) were pretreated for 30 min. with **3b** compound dissolved in DMSO prior to adding WST-1/mPMS (final concentration of 500 μM and 20 μM, respectively). WST-1 reduction measured in real time at 450 nm in a BMG Fluostar microplate reader. Results are averages ± S.E.M. of four independent experiments.



Comp.	R	R_1	R_2	R_3
3a	H	H	H	H
3b	H	Cl	H	H
3c	H	H	Cl	H
3d	H	H	OCH_3	H
3e	H	H	OH	H
3f	H	H	$CO(CH_2)_2COOH$	H
3g	H	H	$N(CH_3)_2$	H
3h	H	H	F	H
3i	H	H	COOH	H
3j	H	H	Br	H
3k	H	H	SO_3H	H
3l	H	H	H	Cl
3m	H	H	OH	CH_3
3n	H	H	OCH_3	CH_3
3o	CH_3	H	H	H
3p	CH_3	H	OCH_3	H
3q	CH_3	H	OH	H

Scheme 1.



SUTENT TAS-202 RITA

Chart 1.

Table 1

Compounds **3**, **7–9**.

Comp.	Formula	M.W.	Method	M.p. or ref.	Starting material ref.
3a	$C_{23}H_{15}N_3O_2$	365.39	1	[a]	
3b	$C_{23}H_{13}Cl_2N_3O_2$	434.28	1	[b]	
3c	$C_{23}H_{13}Cl_2N_3O_2$	434.28	1	[b]	
3d	$C_{25}H_{19}N_3O_4$	425.44	1	[a]	
3e	$C_{23}H_{15}N_3O_4$	397.39	1	335–340	[c]
3f	$C_{31}H_{23}N_3O_8$	565.53	2	210–215 dec.	[d]
3g	$C_{27}H_{25}N_5O_2$	451.52	1	340–350	[e]
3h	$C_{23}H_{13}F_2N_3O_2$	401.37	1	347–350 dec.	[f]
3i	$C_{25}H_{15}N_3O_6$	453.41	2	>360	[g]
3j	$C_{23}H_{13}Br_2N_3O_2$	523.18	2	355 dec.	[h]
3k	$C_{23}H_{15}N_3O_8S_2$	525.52	2	325–330	
3l	$C_{23}H_{13}Cl_2N_3O_2$	434.28	1	332 dec.	
3m	$C_{25}H_{19}N_3O_4$	425.44	1	>360	[i]
3n	$C_{27}H_{23}N_3O_4$	453.49	1	314–315 dec.	[j]
3o	$C_{25}H_{19}N_3O_2$	393.44	1	[a]	
3p	$C_{27}H_{23}N_3O_4$	453.49	1	218–220	[k]
3q	$C_{25}H_{19}N_3O_4$	425.44	1	260–262 dec.	[k]
7a	$C_{20}H_{12}N_4O_4$	372.34	-	>360	
7b	$C_{22}H_{16}N_4O_6$	432.39	-	>360	
8a	$C_{26}H_{20}N_4O_4$	452.46	-	302–305 dec.	
8b	$C_{28}H_{24}N_4O_6$	512.52	-	344–346 dec.	
9a	$C_{22}H_{16}N_4O_2$	368.39	-	355–358 dec.	
9b	$C_{24}H_{20}N_4O_4$	428.44	-	>360	

[a] Ref.12

[b] Ref 13

[c–h] Ref.26–31

[i] Ref.9

[j,k] Ref.32,33

Table 2

Sixty cell panel (growth inhibition, cytostatic and cytotoxic activity of the selected compounds).

Comp [a]	Modes	Leu-kemia	NSCLC	Colon	CNS	Mela-noma	Ova-rian	Renal	Pros-tate	Breast	MG-MID [b]
3a	pGI_{50}	6.63	6.09	6.55	6.24	6.52	6.23	6.24	6.20	6.31	6.34
	pTGI	5.71	5.50	6.11	5.74	6.03	5.75	5.79	5.57	5.70	5.79
	pLC_{50}	-	4.65	5.19	5.15	5.33	5.18	5.27	4.61	4.24	4.85
3b	pGI_{50}	7.04	6.17	6.38	6.22	6.23	6.30	6.37	6.36	6.41	6.34
	pTGI	5.72	5.57	5.30	5.55	5.50	5.55	5.82	5.68	5.48	5.55
	pLC_{50}	4.35	4.46	4.72	4.63	4.80	4.51	5.12	4.80	4.40	4.63
3c	pGI_{50}	6.18	5.76	6.03	6.05	6.16	5.90	5.90	5.82	5.95	5.97
	pTGI	5.31	5.36	5.59	5.56	5.72	5.38	5.53	5.18	5.38	5.47
	pLC_{50}	4.32	4.70	4.82	4.83	5.10	4.75	5.12	4.61	4.64	4.79
3d	pGI_{50}	6.21	5.77	6.10	5.79	6.05	5.95	5.84	5.78	5.83	5.92
	pTGI	5.42	4.97	5.33	4.89	5.40	5.03	5.37	5.03	4.83	5.15
	pLC_{50}	4.24	4.36	4.51	4.20	4.87	4.33	4.62	4.16	4.09	4.40
3e	pGI_{50}	4.76	4.47	4.41	4.39	4.08	4.08	4.46	4.70	4.39	4.40
	pTGI	4.26	-	-	-	-	-	4.16	-	4.07	4.06
	pLC_{50}	4.03	-	-	-	-	-	4.02	-	-	4.01
3f	pGI_{50}	4.81	-	-	4.52	-	-	-	-	-	4.12
	pTGI	-	-	-	4.21	-	-	-	-	-	4.02
3g	pGI_{50}	5.63	5.28	5.17	5.36	5.36	5.12	5.06	5.45	5.22	5.27
	pTGI	5.02	4.36	4.41	4.35	4.52	-	4.30	4.53	4.16	4.39
	pLC_{50}	-	-	-	4.05	4.22	-	-	-	-	4.04
3h	pGI_{50}	5.93	5.14	5.67	5.42	5.69	5.38	5.38	5.46	5.53	5.49
	pTGI	5.11	4.58	5.27	4.94	5.31	4.60	4.89	4.91	4.84	4.93
	pLC_{50}	-	4.10	4.40	4.27	4.44	4.09	4.29	4.13	4.30	4.22
3i	pGI_{50}	5.21	-	-	4.14	-	-	-	-	-	4.14
	pTGI	4.67	-	-	4.06	-	-	-	-	-	4.08
	pLC_{50}	4.46	-	-	-	-	-	-	-	-	4.05
3j	pGI_{50}	5.01	4.96	4.98	5.43	5.00	5.01	5.10	5.19	5.21	5.08

J Med Chem. Author manuscript; available in PMC 2009 September 29.

Comp [a]	Modes	Leu-kemia	NSCLC	Colon	CNS	Mela-noma	Ova-rian	Renal	Pros-tate	Breast	MG-MID[b]
	pTGI	-	4.55	4.61	4.85	4.63	4.59	4.66	4.68	4.74	4.59
	pLC_{50}	-	4.20	4.28	4.39	4.28	4.23	4.29	4.26	4.27	4.25
3k	pGI_{50}	4.03	-	-	-	4.02	-	4.07	-	-	4.01
3l	pGI_{50}	5.84	5.43	5.76	5.65	5.75	5.52	5.97	5.47	5.64	5.69
	pTGI	5.09	4.83	5.31	5.11	5.21	4.71	5.48	4.39	4.87	5.06
	pLC_{50}	4.02	4.24	4.60	4.44	4.67	4.20	4.70	4.15	4.28	4.40
3m	pGI_{50}	4.56	4.07	4.14	-	4.06	4.12	4.17	4.18	4.15	4.16
	pTGI	-	-	-	-	-	-	-	-	4.06	4.01
3n	pGI_{50}	5.36	4.08	4.31	4.05	4.10	4.05	4.28	4.47	4.13	4.29
	pTGI	4.22	-	-	-	-	-	-	-	4.03	4.02
	pLC_{50}	4.04	-	-	-	-	-	-	-	-	-
3o	pGI_{50}	5.51	4.92	5.00	5.12	5.31	5.00	5.00	4.87	5.05	5.09
	pTGI	4.80	4.52	4.55	4.53	4.65	4.53	4.63	4.40	4.48	4.57
	pLC_{50}	4.30	4.30	4.30	4.30	4.36	4.30	4.33	4.30	4.30	4.31
3p	pGI_{50}	5.93	5.61	5.84	5.49	5.98	5.59	5.82	5.68	5.79	5.76
	pTGI	4.42	4.72	5.47	4.83	5.42	4.50	5.28	-	4.70	4.91
	pLC_{50}	-	4.13	4.93	-	5.05	-	4.68	-	4.20	4.36
7a	pGI_{50}	-	4.07	-	4.13	-	4.01	4.08	-	4.07	4.05
8a	pGI_{50}	4.03	4.07	-	4.12	4.06	4.10	4.53	-	4.18	4.13
	pTGI	-	-	-	-	-	-	4.30	-	-	4.04
9a	pGI_{50}	-	4.01	-	4.19	4.26	-	4.15	-	4.19	4.09
9b	pGI_{50}	-	-	-	-	4.14	4.05	-	-	4.10	4.03
Vincristine sulfate[c]	pGI_{50}	7.00	6.60	7.00	6.90	6.80	6.50	6.50	6.90	6.50	6.70
	pTGI	4.80	4.80	5.40	5.20	5.10	4.70	4.70	5.20	5.10	5.00
	pLC_{50}	3.20	3.60	4.10	3.70	3.60	3.50	3.60	3.50	3.50	3.60

[a] Highest conc. = 10^{-4}M unless otherwise reported; only modes showing a value > 4.00 are reported

[b] Mean Graph MIDpoint i.e. the calculated mean panel

[c] Highest conc. = 10^{-3}M

Table 3

Summary of *In Vitro* Growth Data

Breast Cancer Cell Line	3b IC_{50}	MG-132 IC_{50}
MCF-7	0.23 μM	0.3 μM
MDA-MB-231	0.3 μM	0.25 μM
Hs578T	0.4 μM	not done