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Synthesis and Chemical Characterization of 2-Methoxy-N^{10}-substituted Acridones Needed to Reverse Vinblastine Resistance in Multidrug Resistant (MDR) Cancer Cells

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Abstract—In an attempt to find clinically useful modulators of multidrug resistance (MDR), a series of 19 N^{10}-substituted-2-methoxyacridone analogues has been synthesized. 2-Methoxyacridone and its derivatives (**1**–**19**) were synthesized. Compound **1** was prepared by the Ullmann condensation of *o*-chlorobenzoic acid and *p*-anisidine followed by cyclization using polyphosphoric acid. This compound undergoes *N*-alkylation in the presence of phase transfer catalyst (PTC). Stirring of 2-methoxy acridone with 1-bromo-3-chloropropane or 1-bromo-4-chlorobutane in a two-phase system consisting of organic phase (tetrahydrofuran) and 6 N potassium hydroxide in the presence of tetrabutylammonium bromide leads to the formation of compounds **2** and **11** in good yield. *N*-(ω-Chloroalkyl) analogues were found to undergo iodide catalyzed nucleophilic substitution reaction with various secondary amines. Products were characterized by UV, IR, ^{1}H and ^{13}C NMR, mass-spectral data and elemental analysis. The lipophilicity expressed in $\log_{10}$ P and pK_a of compounds have been determined. All compounds were examined for their ability to increase the uptake of vinblastine (VLB) in MDR KBCh^{R}-8-5 cells and the results showed that the compounds **7**, **10**, **12**, and **15**–**19** at 100 μM caused a 1.05- to 1.7-fold greater accumulation of vinblastine than did a similar concentration of the standard modulator, verapamil (VRP). However, the effects on VLB uptake were specific because these derivatives had little effect in the parental drug sensitive line KB-3-1. Steady state accumulation of VLB, a substrate for P-glycoprotein (P-gp) mediated efflux, was studied in the MDR cell line KBCh^{R}-8-5 in the presence and absence of novel MDR modulators. Results of the efflux experiment showed that VRP and each of the modulators (**1**–**19**) significantly inhibited the efflux of VLB, suggesting that they may be competitors for P-gp. From among the compounds examined, 14 except **1**, **2**, **4**, **8**, and **11**, exhibited greater efflux inhibiting activity than VRP. All the 19 compounds effectively compete with [^{3}H] azidopine for binding to P-gp, pointed out this transport membrane protein as their likely site of action. Cytotoxicity has been determined and the IC_{50} values lie in the range 8.00–18.50 μM for propyl and 4–15 μM for butyl derivatives against KBCh^{R}-8-5 cells suggesting that the antiproliferative activity increases as chain length increases from 3 to 4 carbons at N^{10}-position. Compounds at IC_{10} were evaluated for their efficacy to modulate the cytotoxicity of VLB in KBCh^{R}-8-5 cells and found that the modulators enhanced the cytotoxicity of VLB by 5- to 35-fold. Modulators **12**, **14**–**16**, and **19** like VRP, were able to completely reverse the 24-fold resistance of KBCh^{R}-8-5 cells to VLB. Examination of the relationship between lipophilicity and antagonism of MDR showed a reasonable correlation suggesting that hydrophobicity is one of the determinants of potency for anti-MDR activity of 2-methoxyacridones.

Introduction

Multidrug resistance (MDR) is the phenomenon by which tumor cells exhibit an intrinsic resistance or where acquired resistance to one cytotoxic agent results in cross-resistance to many structurally unrelated agents, has been and continues to be a major focus in experimental therapy of human cancer.[1,2] The phenomenon is caused by the overexpression of a 170 kDa transmembrane glycoprotein known as P-glycoprotein (P-gp) encoded by the *mdr1* gene in MDR cells.[3] P-gp has been shown to bind ATP[4,5] and drug analogues,[6,7] has ATPase activity[8] and catalyzes ATP-dependent drug efflux to effectively reduce intracellular accumulation in

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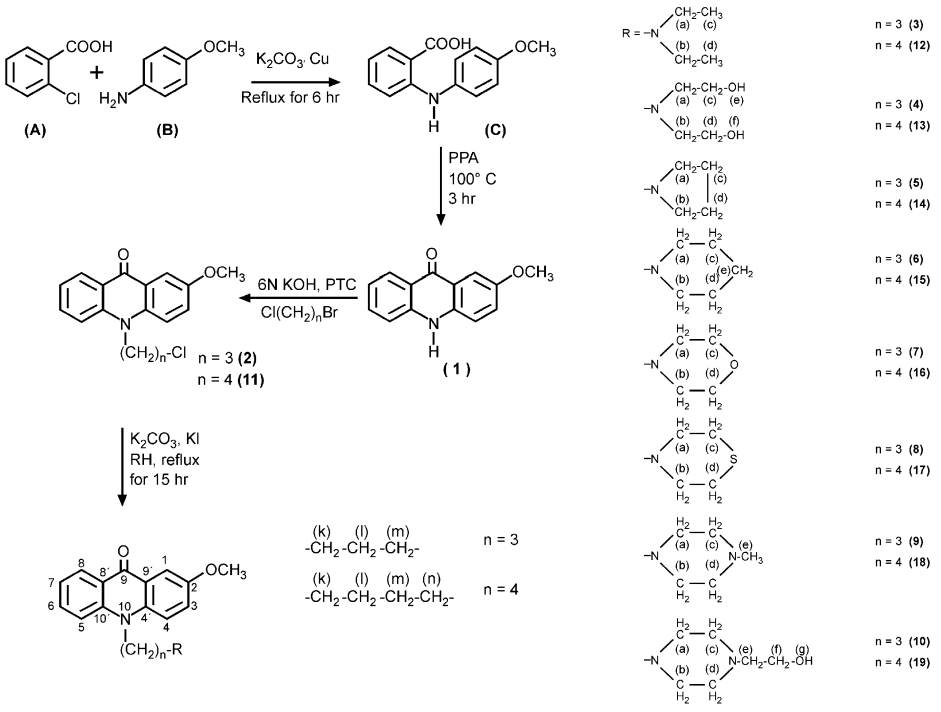


Scheme 1.

resistant cells.[3,9] Many diverse compounds that are capable of modulating P-gp include calcium channel blockers,[10] calmodulin inhibitors,[11] antiarrythmics,[12,13] antimalarials,[14] and other lysosomotropic agents,[15] steroids,[16] antiestrogens,[17] and cyclic peptide antibiotics.[18] They lower the IC_{50} values of a variety of drugs included in the MDR family and they increase intracellular drug concentrations in resistant cells. The mechanisms responsible for this reversal of resistance is believed to be competition between the modulator and cytotoxic drug for binding to the ATP-dependent efflux pump, P-gp.[19,20] The clinical utility of any modulator, however, depends not only on its ability to reverse drug resistance at low concentrations but also on whether it has a low toxicity in vivo. The cardiac toxicity seen during the clinical evaluation of verapamil as a chemosensitizing agent pointed out the need for less toxic modulators.[21,22] Two other antiarrythmic drugs, quinidine and amiodarone, have also entered clinical trials as chemosensitizing agents[23] and both drugs have produced a number of adverse clinical side effects.[24] While a number of pharmacological agents have been shown to reverse MDR in vitro, there remains a need to identify more potent, more specific and less toxic chemosensitizers for clinical use.

The reversal of MDR can be obtained by treatment with numerous classes of lipophilic positively, charged drugs known as MDR inhibitors. Latest studies have raised hopes that addition of MDR inhibitors to cytotoxics might improve chemotherapy success rate. The design of potent MDR inhibitors devoid of other pharmacological activities has thus become a desirable goal to test the MDR reversal hypothesis in the clinic. In the course of a chemical program aimed at identifying potent MDR inhibitors, Hyafil et al.[25] have identified a series of acridone carboxamide derivatives and from among them one derivative, GF120918,[26] was found to be extremely potent in reversing the MDR. In two animal models of MDR, the P388/DOX leukemia and the C26 colon carcinoma, a single iv dose of GF120918 restores the antiproliferative action of doxorubicin. Further, in an ongoing program, the investigators' group has already demonstrated a novel acridone derivative [1,3-bis (9-oxoacridin-10-yl)propane], as a potent and poorly reversible modulator of P-glycoprotein mediated vinblastine transport.[27] This interesting data has inspired the authors to synthesize and describe the properties of 19 2-methoxyacridones as potential modulators of MDR against $KBCh^R$-8-5 cells.

Chemistry

2-Methoxyacridone (**1**) and its derivatives (**2**–**19**) were prepared by the synthetic route as outlined in Scheme 1. Compound **1** was prepared by the Ullmann condensation of *o*-chlorobenzoic acid and *p*-anisidine to form 4′-methoxydiphenylamine-2-carboxylic acid (**C**). The reactants were heated preferably at reflux in the presence of copper powder and potassium carbonate in isoamylalcohol medium. The 4′-methoxydiphenylamine-2-carboxylic acid (**C**) was cyclized with sulfuric acid at 100 °C on a water-bath to form 2-methoxyacridone (**1**) (60%) and sulphonated 2-methoxyacridone (40%) as evidenced by TLC. When cyclization was carried out with polyphosphoric acid instead of sulfuric acid on a water-bath at 100 °C only single product of 2-methoxyacridone (**1**) with better yield (90%) was obtained.

The weakly basic nature of nitrogen atom of the acridone nucleus usually resists to undergo *N*-alkylation with alkyl halides. However, it can be achieved in the

presence of basic condensing agents like sodium amide or sodium hydride. The general procedure for preparing *N*-alkyl derivative consists of condensation of acridone with requisite alkyl halide in the presence of a strong acid binding agent like sodium amide in anhydrous aromatic solvents such as toluene or benzene. The reaction of 2-methoxyacridone with mixed chlorobromoalkanes in the presence of sodium amide in anhydrous toluene under reflux conditions gave N^{10}-(chloroalkyl)-2-methoxyacridone, but the yield was low, besides requiring drastic experimental conditions. However, this compound undergoes *N*-alkylation in the presence of PTC more easily compared to previously described preparative procedure.

Stirring of 2-methoxyacridone (**1**) at room temperature with alkylating agent (Br-$(CH_2)_3$-Cl or Br-$(CH_2)_4$-Cl) in a two-phase system consisting of an organic solvent (tetrahydrofuran) and a 6 N aqueous potassium hydroxide solution in the presence of tetrabutylammonium bromide [$(n\text{-}C_4H_9)_4N^+Br^-$] leads to the formation of the compound **2** or **11** in good yield. Here, ammonium salt transports hydroxide ion from aqueous phase to organic phase where the actual reaction takes place. These results are interpreted by deprotonation of **1** by the OH^-, transferred by the catalyst into the organic layer. The anion formed may be regarded as phenolate stabilized anion, which subsequently undergoes alkylation to form the aromatized system.

Iodide catalyzed nucleophilic substitution of the N^{10}-propyl or N^{10}-butylchloride of 2-methoxyacridone (**1**) with various secondary amines (*N*,*N*-diethylamine, *N*,*N*-diethanolamine, pyrrolidine, piperidine, morpholine, thiomorpholine, 1-methylpiperazine and (β-hydroxyethyl)piperazine) by refluxing for different times in the presence of potassium carbonate in acetonitrile gave the free bases **3**–**10** and **12**–**19**.

All the products were separated and purified by column chromatography or recrystallization method and dried under high vacuum for more than 12 h. The purified compounds were characterized by UV, IR, ^1H and ^{13}C NMR, mass spectral, and elemental analysis.

Pharmacological Results and Discussion

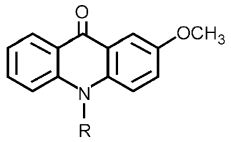
pK_a and lipophilicity effects

The effectiveness of any agent as an MDR modulator will depend in part on its ability to accumulate in cells. The 2-methoxyacridones are weak bases and able to exist in both charged (protonated) and uncharged (unprotonated) forms. The unprotonated or neutral form of compounds will be highly membrane permeable and able to diffuse freely and rapidly across biological membranes. In contrast, the protonated form would be at least an order of magnitude less membrane permeable and diffuse across membranes at a much reduced rate.[28] In addition, if the unprotonated form of the molecule diffuses across the membrane and enters an acidic compartment within the cell, it will rapidly become protonated and unable to diffuse out of the cell. The magnitude of the biological activity depends on pK_a of compounds besides other factors. For the series of compounds examined, the pK_a values (Table 1) ranging from 8.3 to 9.6 lie closer to physiological pH, which may suggest that these compounds accumulate in MDR cells as free bases rather than in protonated form. The lipophilicity data varying from 1.3 to 2.3, expressed in $\log_{10}$P for 19 2-methoxyacridones are given in Table 1. Within the series, all compounds are highly lipophilic at pH 7.4 and it is expected that they will accumulate rapidly into cells. Analysis of the relationship between lipid solubility ($\log_{10}$P) and the effectiveness of the modulators to increase VLB accumulation in drug-resistant KBCh^R-8-5 cells showed a good correlation (R = 0.78, data not shown). The major outliyer in this analysis was that the degree of lipophilicity of each drug would seem to be important and it is one of the determinants of potency for the P-gp modulating activity of 2-methoxyacridones.

Effect of 2-methoxy-N^{10}-substituted acridones on the accumulation of VLB in KBCh^R-8-5 cells

In order to explore the potential of 2-methoxy-N^{10}-substituted acridones to enhance the uptake of VLB, the effect of 19 compounds at 100 μM was determined in MDR KBCh^R-8-5 cells. As shown in Table 1, compounds **1**–**19** at 100 μM concentration exhibited significant VLB accumulation enhancing effect (7- to 20-fold relative to control) compared to a standard modulator verapamil (VRP) (11.9-fold). Eleven compounds **1**–**6**, **8**, **9**, **11**, **13**, and **14** were found to possess less VLB enhancing effect (7- to 11.2-fold) compared to VRP (11.9-fold) relative to control. Remaining eight compounds (**7**, **10**, **12**, and **15**–**19**) caused a 1.05- to 1.7-fold greater accumulation of VLB than did a similar concentration of VRP. The enhancement of VLB uptake was specific for MDR cell line since all the compounds (**1**–**19**) had very little effect in sensitive KB-3-1 cells (data not shown). A similar effect was observed on the effect of phenoxazines on the accumulation of VLB in drug sensitive KB-3-1 cells.[29] The VLB uptake enhancing effects with respect to percentage of control are in the range 842–1278% for propyl derivatives and 996–1998% for butyl derivatives suggesting that the butyl derivatives seem to rank the list of these compounds. Comparative study of the VLB uptake data of the modulators within the propyl derivatives revealed that all the propyl compounds except **7** and **10**, exhibited almost the same uptake enhancing effect. Similar comparative study within the butyl derivatives on the uptake of VLB into KBCh^R-8-5 cells revealed that the compounds follow the order: **11** < **13**≈**14** < **12** < **17**≈**18** < **16** < **15** < **19**. Further, the effects of varying concentrations of modulators **5**, **6**, **7**, **8**, **9**, **10**, **12**, **15**, **19**, or VRP on the uptake of VLB in MDR cells were studied. The KBCh^R-8-5 cells were exposed for 2 h to 49.9 nM [^3H] VLB in the absence or presence of 10 μM, 25 μM, 50 μM, or 100 μM concentration of the above nine compounds or VRP and the intracellular concentration of VLB in picomoles/10^6 cells was calculated. The results revealed that all the modulators at 10 μM exhibited the least VLB enhancing effect whereas at 25, 50, and 100 μM,

Table 1. Effect of 2-methoxy-N^{10}-substituted acridones on accumulation of vinblastine in KBCh^{R}-8-5-cells



Compd	R	pK_a	$\log_{10}$P[a]	Vinblastine uptake (% control)[b] ± SEM
1	-H	8.29	2.11	699 ± 2.11
2	-CH_2-CH_2-CH_2-Cl	8.80	1.32	842 ± 1.32
3	-CH_2-CH_2-CH_2-N(CH_2-CH_3)(CH_2-CH_3)	5.35 9.16	1.35	866 ± 1.35
4	-CH_2-CH_2-CH_2-N(CH_2-CH_2-OH)(CH_2-CH_2-OH)	4.69 9.25	1.30	900 ± 1.30
5	-CH_2-CH_2-CH_2-N(CH_2-CH_2)(CH_2-CH_2)	5.20 9.29	1.34	933 ± 1.34
6	-CH_2-CH_2-CH_2-N(CH_2-CH_2)(CH_2-CH_2)CH_2	5.34 9.30	1.52	927 ± 1.52
7	-CH_2-CH_2-CH_2-N(CH_2-CH_2)(CH_2-CH_2)O	4.77 9.20	1.70	1278 ± 1.70
8	-CH_2-CH_2-CH_2-N(CH_2-CH_2)(CH_2-CH_2)S	4.95 9.30	1.30	900 ± 1.30
9	-CH_2-CH_2-CH_2-N(CH_2-CH_2)(CH_2-CH_2)N-CH_3	5.27 9.15	1.42	996 ± 1.42
10	-CH_2-CH_2-CH_2-N(CH_2-CH_2)(CH_2-CH_2)N-CH_2-CH_2-OH	5.24 9.09	1.75	1245 ± 1.75
11	-CH_2-CH_2-CH_2-CH_2-Cl	8.82	1.40	996 ± 1.40
12	-CH_2-CH_2-CH_2-CH_2-N(CH_2-CH_3)(CH_2-CH_3)	5.43 9.36	1.85	1301 ± 1.85
13	-CH_2-CH_2-CH_2-CH_2-N(CH_2-CH_2-OH)(CH_2-CH_2-OH)	4.78 9.30	1.60	1099 ± 1.60
14	-CH_2-CH_2-CH_2-CH_2-N(CH_2-CH_2)(CH_2-CH_2)	5.24 9.60	1.65	1116 ± 1.65
15	-CH_2-CH_2-CH_2-CH_2-N(CH_2-CH_2)(CH_2-CH_2)CH_2	5.21 9.60	2.30	1919 ± 2.30
16	-CH_2-CH_2-CH_2-CH_2-N(CH_2-CH_2)(CH_2-CH_2)O	4.93 9.10	2.00	1673 ± 2.00

(*continued on next page*)

Table 1 (*continued*)

Compd	R	pK_a	$\log_{10}$P[a]	Vinblastine uptake (% control)[b] ± SEM
17	-CH_2-CH_2-CH_2-CH_2-N(-CH_2-CH_2-)(-CH_2-CH_2-)S	5.24 9.25	1.90	1410 ± 1.90
18	-CH_2-CH_2-CH_2-CH_2-N(-CH_2-CH_2-)(-CH_2-CH_2-)N-CH_3	5.65 9.21	1.90	1410 ± 1.90
19	-CH_2-CH_2-CH_2-CH_2-N(-CH_2-CH_2-)(-CH_2-CH_2-)N-CH_2-CH_2-OH	5.27 9.38	2.30	1998 ± 2.30
Verapamil		ND	ND	1190 ± 1.72

ND, not determined.
[a]Octanol/water partition coefficient.
[b]$\left(\frac{\text{Vinblastine uptake with modulator}}{\text{Vinblastine uptake without modulator}}\right) \times 100$. Compounds were tested at 100 μM. All values represent the mean of two separate experiments and each experiment was done in triplicate.

the modulators exhibited a significant increase on the accumulation of VLB suggesting that the uptake of VLB into KBCh^R-8-5 cells is dependent on the concentration of modulator. The most effective modulator is **19** allowing an almost 20-fold increase in accumulation of VLB relative to control. The presence of an N^{10}-substitution seems to be necessary to optimize the activity in 2-methoxyacridones. Comparison of the derivatives for their ability to potentiate the uptake of VLB in KBCh^R-8-5 cells revealed that they follow the order N^{10}-butyl > N^{10}-propyl, although there are one or two exceptions.

Effect of substituted acridones on the active outward transport of vinblastine from KBCh^R-8-5 cells

Decreased uptake of cytotoxics in many MDR cells is attributed to increased efflux mediated by P-gp. To determine whether the increase in VLB accumulation upon coincubation with 2-methoxyacridones (**1**–**19**) was due to a slowing of P-gp mediated VLB efflux, KBCh^R-8-5 cells were loaded with 49.9 nM [^{3}H] VLB for 2 h in the absence of modulators and resuspended in [^{3}H] VLB free buffer with or without 100 μM modulators (**1**–**19**) or VRP for an additional 2 h at room temperature. The cell associated radiolabel remaining after a 2 h efflux was determined and calculated as a percentage of the VLB present after loading. The data on the fraction of VLB remaining after 2 h are given in Table 2. Results of the efflux experiment showed that VRP and each of the modulators (**1**–**19**) significantly inhibited the efflux of VLB, suggesting that they may be competitors for P-gp. After incubation of the cells for 2 h in the absence of modulator, more than 80% of VLB was lost from the cells, whereas 56–86% of VLB in the presence of modulators (**1**–**19**) or 67% of VLB in the presence of VRP was retained in the cells. From among 19 modulators, 14 compounds except **1**, **2**, **4**, **8**, and **11**, exhibited greater efflux inhibiting activity than VRP. Possibly all the modulators (**1**–**19**) are able to both enhance [^{3}H] VLB accumulation and inhibit significantly the efflux of VLB from MDR KBCh^R-8-5 cells.

The time-course of VLB efflux from MDR cells was also done (Fig. 1). The cells were loaded with 49.9 nM [^{3}H] VLB for 2 h at room temperature and washed and the retained radioactivity was measured at the indicated times. When efflux was examined in the absence of modulator, less than 20% of the cellular VLB remained after 2 h. When a similar experiment was performed, but with 100 μM concentration of VRP or compound **19** present continuously, about 67% or 80% of the cell-associated VLB remained at the end of a 2 h efflux period. This data suggests that the 2-methoxyacridones like VRP, are able to inhibit P-gp mediated VLB efflux from KBCh^R-8-5 cells.

Table 2. Effect of 2-methoxy-N^{10}-substituted acridones on the cellular retention of VLB in KBCh^R-8-5 cells after 2 h efflux

Compd	Cellular retention (%) of VLB after 2 h efflux
1	56.00
2	63.00
3	77.00
4	62.00
5	68.50
6	78.40
7	77.15
8	57.30
9	72.40
10	71.00
11	63.00
12	86.20
13	79.60
14	79.80
15	81.00
16	82.00
17	84.00
18	84.00
19	80.00
Verapamil	67.00
Control	19.50

All values represent the mean of two separate experiments with a SD of less than 10% of the mean; each experiment was done in triplicate.

To investigate whether 2-methoxyacridones interact with P-gp by photolabeling this protein with [^{3}H] azidopine

Cornwell et al.[30] originally showed that P-gp of the MDR KB cells is specifically labeled with VLB analogues and that VRP blocked the specific labeling. A dihydropyridine analogue has been found to specifically interact with P-gp by a photoaffinity labeling assay. The reversing of drug resistance by chemosensitizers seems to be correlated with the inhibition of [^{3}H] azidopine photolabeling of P-gp. Photoaffinity labeling with [^{3}H] azidopine was not detected under our experimental conditions when used membranes prepared from drug-sensitive KB-3-1 cells or from KBCh^R-8-5 cells, which have a low level of drug resistance (data not shown). A highly VLB resistant and P-gp expressing KB cell subline KB-V1 was therefore used for [^{3}H] azidopine competition studies.

The VLB transport and cell survival data suggest that the modulatory effect of 2-methoxyacridones is through an interaction with P-gp. To confirm this suggestion the competition between [^{3}H] azidopine and 19 2-methoxyacridones (**1**–**19**) was determined. At 100 μM 2-methoxyacridones inhibited the labeling of P-gp by [^{3}H] azidopine. The binding of [^{3}H] azidopine to P-gp after inhibition by acridones expressed in percentage of control (no competitor) is as follows: **1** by 65%, **2** by 70%, **3** by 60%, **4** by 55%, **5** by 45%, **6** by 40%, **7** by 20%, **8** by 54%, **9** by 50%, **10** by 10%, **11** by 65%, **12** by 19%, **13** by 20%, **14** by 24%, **15** by 12%, **16** by 18%, **17** by 20%, **18** by 19%, **19** by 15%, and VRP by 60%. Comparison of the data on the competition between [^{3}H] azidopine and each acridone modulator at 100 μM concentration revealed that the ability of all the compounds, except compounds **1**–**3** and **11**, to inhibit the [^{3}H] azidonpine labeling of P-gp, is greater than that of the standard modulator VRP by 1.1- to 5-fold. If a modulator inhibits labeling by the probe of interest, then it is said that this modulator probably functions by competing for the drug binding site on the protein. The fact that all the compounds (**1**–**19**) have reduced the photoaffinity labeling of azidopine appreciably, the results predict that the modulators compete for azidopine for binding to P-gp. Careful evaluation of the data of the modulators showed that butyl derivatives have exhibited greater competition than that of propyl derivatives suggesting that this is due to enhanced lipophilicity. From among the compounds tested, one propyl derivative (**10**) and two butyl derivatives (**15** and **19**) exhibited the maximum competition (85–88%) and the remaining butyl derivatives except (**11**), quoted to be around 76–80% competition. Comparison of the inhibition data has revealed that those modulators, which have exhibited greater VLB accumulation effect and maximum VLB efflux preventing ability from MDR cells, have exhibited greater competition for azidopine labeling, suggesting that the activity of 2-methoxyacridones (**1**–**19**) may be mediated through P-gp-dependent mechanism.

Effect of 2-methoxy-N^{10}-substituted acridones on inhibition of cellular proliferation

The cytotoxicity of 2-methoxyacridones was examined by incubating the drug-sensitive (KB-3–1) cells continuously for 7 days with a range of concentrations up to 100 μM. Examination of the IC_{50} values has revealed that the compounds are relatively nontoxic (data not given).

The cytotoxicity of each of the 19 2-methoxyacridones (**1**–**19**) was examined in MDR KBCh^R-8-5 cells. The concentration of modulators (**1**–**19**) that reduced colony formation by 10 and 50% (IC_{10} and IC_{50}) were determined from concentration percent survival curves and the results are given in Table 3. The IC_{10} and IC_{50} values for 19 modulators (**1**–**19**) lie, respectively, in the range 0.5–5 and 4.0–25.0 μM for KBCh^R-8–5 cells. Careful examination of IC_{50} values for -N^{10}-chloropropyl (8.0-18.5 μM) and -N^{10}-chlorobutyl (4.0–9.3 μM) derivatives against KBCh^R-8-5

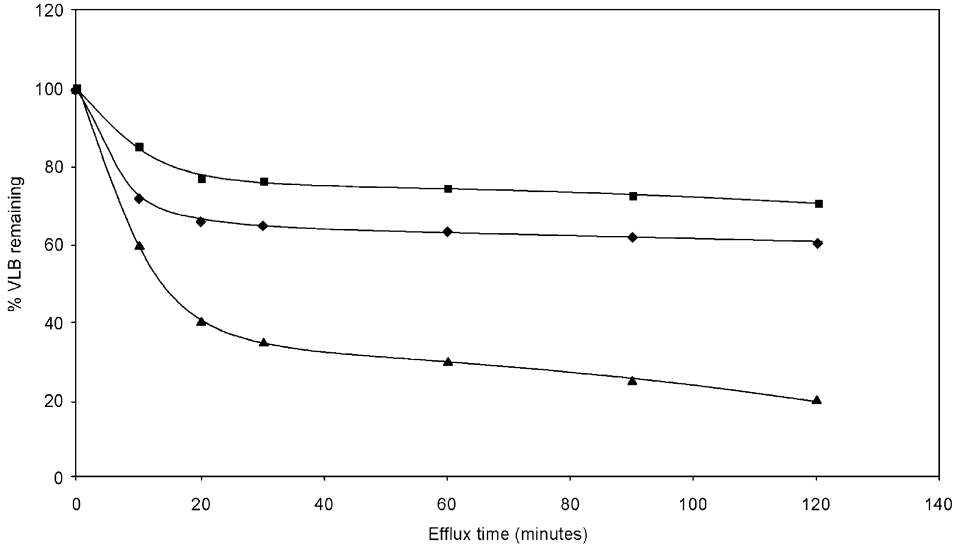


Figure 1. Effect of modulator **19** and verapamil on the outward transport of [^{3}H]vinblastine from KBCh^R-8-5 cells. Efflux studies were done in the presence of vehicle control, 0.1% DMSO (▲), modulator **19** (■) or verapamil (◆) as described in Experimental section.

cells revealed that antiproliferative activity largely increased as the chain length increased from 3 to 4 suggesting that the hydrophobicity plays an important role on the biological activity. Increasing the distance between the ring nucleus and amino group increased the antiproliferative activity of these compounds. Comparison of the cytotoxicity of the butyl derivatives has shown that the cell killing potency follows the order: **19** > **16**≈**15**≈**13** > **14** > **12**≈**18** > **17** > **11** against KBCh^R-8–5 cells. The structural features required to cause a maximum antiproliferative activity include a hydrophobic acridone nucleus and a piperazinylamine with a *para*-CH_3 group, joined by a four alkyl bridge to the nucleus. The structural features required within the series to cause a maximum antiproliferative activity in KBCh^R-8–5 cells include hydrophobic acridone ring nucleus with a-OCH_3 substitution at C-2 and a side-chain tertiary cationic amino group that is separated from the aromatic ring by at least three to four carbons. However, it is not possible to draw conclusions about the correlation between structure and antiproliferative activity from these studies.

Table 3. Cytotoxicity of 2-methoxy-N^{10}-substituted acridones in human KBCh^R-8-5 cell line

Compd	IC_{10} (μM)	IC_{50} (μM)
1	1.0	25.0
2	1.5	18.0
3	1.4	12.8
4	1.8	10.8
5	0.9	12.5
6	1.2	14.5
7	5.0	11.0
8	1.3	18.5
9	4.0	15.0
10	1.4	8.0
11	1.50	15.0
12	1.50	8.0
13	0.65	5.0
14	0.60	7.0
15	0.70	5.0
16	0.90	5.0
17	0.50	9.3
18	1.00	8.0
19	0.50	4.0
Verapamil	[30]	[55]

IC_{10} and IC_{50} are the concentrations (μM) required to produce 10% and 50% reduction respectively, in clonogenic survival of KBCh^R-8–5 cells under the conditions described in the Experimental section.

Sensitization of drug-resistant KBCh^R-8-5 cells by 2-methoxy-N^{10}-substituted acridones

We have evaluated the ability of 19 compounds to modulate the cytotoxicity of VLB in MDR cells. Cells (KB-3-1 or KBCh^R-8-5) were exposed continuously to 0–100 nM VLB for 7 days in the absence and presence of IC_{10} concentrations of 2-methoxyacridone modulators (**1**–**19**). The concentration response curves were determined by clonogenic assay, and the IC_{50} and fold-potentiation of VLB cytotoxicity are summarized in Table 4. The IC_{50} values for VLB against KBCh^R-8-5 cells in the presence of IC_{10} of modulator (**1**–**19**) lie in the range of 2–14 nM. Examination of IC_{50} values of VLB in the presence of propyl derivatives (4–12 nM) or butyl derivatives (2–6 nM) of acridone has revealed that the butyl derivatives have sensitized the MDR KBCh^R-8-5 cells to a greater extent presumably due to increased hydrophobicity. Comparative study of the abilities of the modulators to potentiate the cytotoxicity of VLB has revealed that the modulator **19** demonstrated the greatest effect followed by **16**, **14**, **12**, **15**, **18**, and so on. Only five 2-methoxyacridones (**12**, **14**, **15**, **16**, and **19**), like VRP, were able to completely reverse the 24-fold resistance of KBCh^R-8-5 cells to VLB. The IC_{50} values for continuous exposure to VLB was 3 nM in KB-3-1 and 72 nM in KBCh^R-8-5 cells in the absence of modulating agent. The most effective modulators (**19**, **16**, and **12**) in KBCh^R-8-5 cells were subsequently tested in KB-3-1 and all three were shown to cause a small sensitization (2- to 3-fold) of the drug-sensitive line to VLB. However, a similar degree of sensitization was also found when the classical MDR modulator, VRP (3.5-fold) was used. The influence of alkyl bridge length connecting the acridone nucleus to the amino group was examined. Increasing the distance between the ring nucleus and the amino group from three to four carbons showed appreciable difference in the antiproliferative and anti-MDR effects of these compounds. Further, a careful analysis of the relationship between hydrophobicity and antagonism of MDR showed a reasonable correlation (Fig. 2). Thus, the degree of lipophilicity is one of the determinants of potency for anti-MDR activity of 2-methoxyacridones.

Table 4. Effect of 2-methoxy-N^{10}-substituted acridones on the potentiation of vinblastine cytotoxicity in drug resistant KBCh^R-8-5 cells

Compd[a]	VLB IC^b_{50} (nM)	FP[c]	Compd[a]	VLB IC^b_{50} (nM)	FP[c]
1	14.0	5.0	11	6.00	11.7
2	7.5	9.0	12	2.14	comp.[d]
3	8.0	9.0	13	3.80	18.4
4	7.0	10.0	14	2.10	comp.
5	8.0	9.0	15	2.20	comp.
6	7.0	10.0	16	2.10	comp.
7	4.0	17.5	17	5.00	14.0
8	12.0	5.8	18	3.00	23.33
9	7.5	9.0	19	2.00	comp.
10	4.0	17.5			

[a]Modulators used at the IC_{10} concentration (Table 3).
[b]Concentration of modulators required for 50% reduction of cells compared to controls.
[c]Fold-potentiation.
[d]comp., Complete reversal of VLB resistance.

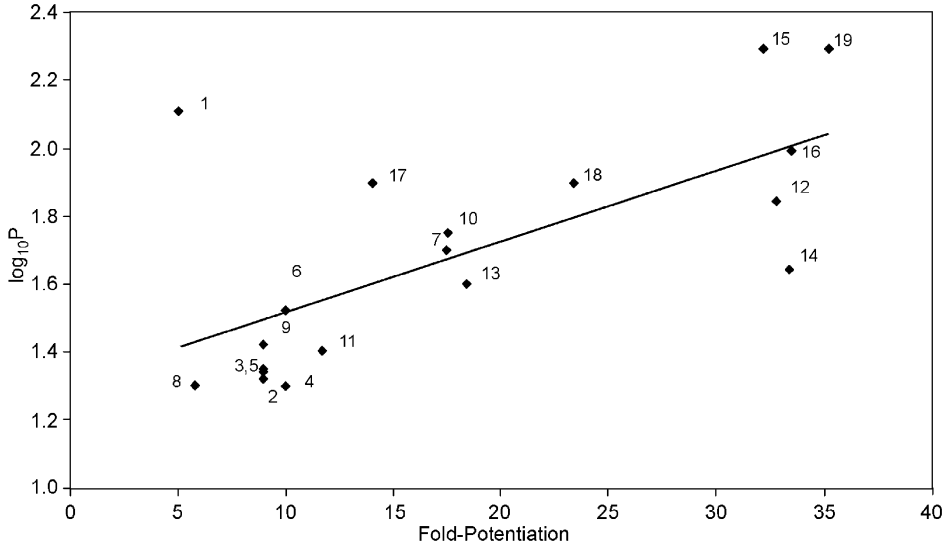


Figure 2. Correlation between the $\log_{10}$ P of 2-methoxy-N^{10}-substituted acridones (**1**–**19**) and antagonism of MDR in KBCh^{R}-8-5 cells.

In summary, we have synthesized a series of novel acridones that showed significant anti-MDR activity at their IC_{10} values against KBCh^{R}-8-5 cells. Five compounds (**12**, **14**–**16**, and **19**) were able to reverse completerly the 24-fold resistance of KBCh^{R}-8-5 cells to vinblastine. Based on the promissing results, the compounds are considered to be potential lead molecules for the future development of clinically useful chemosensitizers.

Experimental

General

Reactions were monitored by TLC. Column chromatography utilized silica gel Merck Grade 60 (230–400 mesh, 60 Å). Melting points were recorded on a Tempirol hot-stage with microscope and are uncorrected. UV spectra were recorded in MeOH on a Shimadzu-UV-1601 spectrophotometer; IR spectra were recorded on a Perkin–Elmer Model 1320 spectrometer as KBr pellets. Elemental analyses were performed at Central Drug Research Institute, Lucknow, India. Found values are within 0.4% of theoretical values unless otherwise noted.

^{1}H and ^{13}C NMR spectra were recorded in DMSO solution in a 5-mm tube on a Bruker drx 500 Fourier transform spectrometer with tetramethylsilane as internal standard. Chemical shifts are expressed as δ (ppm) values. The spectrometer was internally locked to the deuterium frequency of the solvent. To obtain molecular weight information, acridone derivatives were analyzed by electro spray ionization (ESI) and electron ionization (EI) mass spectrometry. In ESI, the sample solution is injected into a continuous stream of a solution that consists of 1:1 mixture aq 0.1% trifluoroacetic acid and acetonitrile. In this study, a single quadrupole mass spectrometer (platform II, micromass) was employed to analyse acridones. ESI is a gentle mode of ionization. It mainly produces protonated ions of the analyte molecules and no fragmentation provided the sampling cone voltage <30 V. In order to reduce the thermally induced fragmentation, the temperature of the ion source was adjusted to 60 °C. The quadrupole was scanned in the mass range of 50 to 450 Da. In the EI technique used an AutoSpec Q (VG Analytical, Manchester, UK) hybrid tandem mass spectrometer of E_1BE_2-qQ geometry (where E is an electric sector, B a magnetic sector, q an rf-only quadrupole and Q a quadrupole mass analyzer). Only the front end (i.e., E_1BE_2) was used for this study. The data accumulation and manipulation were done under digital Vax Station 3100-based Opus Software. Electron ionization-ionizing energy, 70 eV; emission current, 100 μA; source temperature, 100 °C; mass resolution 2000; accelerating potential, 8000 V, mass range 50–500 Da; spectral scan rate, 5 s/decade; samples were introduced via direct insertion probe, which was not heated; the only source of heat to the sample was through contact with the ion source.

Materials

All the chemicals and supplies were obtained from standard commercial sources unless otherwise indicated. DMEM media, Hank's balanced salt and trypsin–EDTA were purchased from Imperial (UK). Vinblastine sulfate was purchased from Cetus Corporation (Emeryville, CA, USA). RPMI-1640 medium with glutamine and without sodium bicarbonate and sodium pyruvate were purchased from Gibco BRL (Grand Island, NY, USA). [^{3}H] vinblastine (sp. act. 9.4 Ci/mmol) was purchased from Amersham Pharmacia Biotech., UK, Ltd. Verapamil hydrochloride, colchicine, dimethyl sulfoxide were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Synthesis

Preparation of 4′-methoxydiphenylamine-2-carboxylic acid (C). Ullmann condensation. To a mixture of *o*-chlorobenzoic acid (**A**) (12.5 g, 0.08 mol), *p*-anisidine

(**B**) (9.84 g, 0.08 mol) and copper powder (0.2 g) in 60 mL of isoamylalcohol, dry potassium carbonate (12 g) was slowly added and the contents were allowed to reflux for 6 h on an oil bath at about 160 °C. The isoamylalcohol was removed by steam distillation and the mixture poured into 1 L of hot water and acidified with concentrated hydrochloric acid. The bluish black precipitate which formed was filtered, washed with hot water and collected. The crude acid was dissolved in aq sodium hydroxide solution, boiled in the presence of activated charcoal and filtered. On acidification of the filtrate with concentrated hydrochloric acid, bluish black precipitate of (**C**) was obtained which was washed with hot water and recrystallized from aq methanol to give greenish yellow needles (14 g, 72%, mp 187 °C). MS (*m*/*z*) 243 ($M^{+\cdot}$).

2-Methoxyacridone (1). Twelve grams of **C** were taken in a round-bottom flask to which was added 120 g of polyphosphoric acid. Shaken well and heated on a water bath at 100 °C for about 3 h. Appearance of yellow color indicated the completion of the reaction. Then, it was poured into 1 L of hot water and made alkaline by liquor ammonia. The yellow precipitate which formed was filtered, washed with hot water, and collected. The sample of 2-methoxyacridone (**1**) was recrystallized from methanol:1 N potassium hydroxide solution [60 + 40 mL] (10 g, 90%, mp 282–283 °C). Further, the purity was checked on TLC plate using the solvent system chloroform:methanol [24 + 1 mL] and the purified product was characterized by spectral methods. UV λ_{max} (∈) (MeOH) 211 (16,824), 250 (40,472), 269 (38,085), 394 (7477) nm. IR 3436, 2987, 2940, 1636, 1533, 1605, 1476, 1502, 1398, 1372, 1238, 1166, 1036, 829, 762, 705 cm^{-1}. ^1H NMR (DMSO-d_6) δ 7.21–8.23 (m, 7H, Ar-H, H_1, H_3, H_4, H_5-H_8), 3.85 (s, 3H, OCH_3), 11.73 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ 115.12 (C_1), 154.28 (C_2), 124.56 (C_3), 105.26 (C_4), 117.68 (C_5), 133.33 (C_6), 121.33 (C_7), 126.26 (C_8), 176.46 (C_9), 136.10 ($C_{4'}$), 140.83 ($C_{10'}$), 119.57 ($C_{8'}$), 121.01 ($C_{9'}$), 55.68 (OCH_3). MS *m*/*z* (%) 226 $(M+H)^+$, 100. Anal. ($C_{14}H_{11}NO_2$) C, H, N.

Synthesis of N^{10}-alkylated acridones via phase-transfer catalysis

10-(3′-Chloropropyl)-2-methoxyacridone (2). One gram (4.44 mmol) of 2-methoxyacridone was dissolved in 20 mL of tetrahydrofuran and 25 mL of 6 N potassium hydroxide and 0.74 g of tetrabutylammonium bromide (2.3 mmol) was added to it. The reaction mixture was stirred at room temperature for 30 min. Added 1-bromo-3-chloropropane (11.5 mmol) slowly into the reaction mixture and stirred at room temperature for 24 h. Tetrahydrofuran was evaporated and the aq layer extracted with chloroform. The chloroform layer was washed with water and the organic layer dried over anhydrous sodium sulfate and rotavaporated. The crude product was purified by column chromatography using the solvent system chloroform–acetone (8 + 1 mL) to give yellow crystals of 10-(3′-chloropropyl)-2-methoxyacridone (**2**) (1.12 g, 51%, mp 130 °C). UV λ_{max} (∈) (MeOH) 213 (13,262), 252 (44,645), 271 (36,241), 399 (8652) nm. IR 3446, 3010, 2945, 1638, 1605, 1506, 1475, 1367, 1274, 1175, 1061, 812, 780, 761 cm^{-1}. ^1H NMR (DMSO-d_6) δ 7.73–8.78 (m, 7H, Ar-H, H_1, H_3, H_4, and H_5-H_8), 3.74 (s, 3H, OCH_3), 3.73 (m, 2H, H_m), 3.71 (m, 2H, H_k), 2.66 (m, 2H, H_l); ^{13}C NMR (DMSO-d_6) δ 110.17 (C_1), 157.91 (C_2), 125.01 (C_3), 108.79 (C_4), 119.21 (C_5), 137.02 (C_6), 123.07 (C_7), 126.21 (C_8), 179.9 (C_9), 138.0 ($C_{4'}$), 140.10 ($C_{10'}$), 121.17 ($C_{8'}$), 121.42 ($C_{9'}$), 59.33 (OCH_3), 46.54 (C_k), 33.56 (C_l), 46.89 (C_m). MS *m*/*z* (%) 301 ($M^{+\cdot}$, 100), 238 (70), 224 (35), 210 (25), 195 (25), 167 (30), 182 (10), 153 (10), 77 (5), 41 (25).

10-[3′-(*N*-Diethylamino)propyl]-2-methoxyacridone (3). Compound **2** (1.11 g, 3.68 mmol) was dissolved in 100 mL of anhydrous acetonitrile and 1.57 g of KI, 2.54 g of K_2CO_3 and 1.17 g (16.02 mmol) of *N*,*N*-diethylamine were added. The mixture was refluxed for 16 h, and the contents were cooled, diluted with water and extracted with chloroform. The chloroform layer was washed thrice with water, dried over anhydrous sodium sulfate and evaporated to give an oily product. The oily residue was purified by column chromatography using the solvent system chloroform–acetone (8 + 1 mL) to give a light-yellow oil of 10-[3′-(*N*-diethylamino)propyl]-2-methoxyacridone (**3**). An acetone solution of the free base was treated with ethereal hydrochloride to give the hydrochloride salt, which was dried over high vacuum to obtain pure solid (**3**) (0.81 g, 40%, mp 91–93 °C). UV λ_{max} (∈) (MeOH) 212 (8828), 252 (33,359), 271 (28,789), 397 (6562) nm. IR 3415, 2985, 2400, 1646, 1567, 1544, 1492, 1144, 1072, 820, 796 cm^{-1}. ^1H NMR (DMSO-d_6) δ 7.34–8.38 (m, 7H, Ar-H, H_1, H_3, H_4, H_5-H_8), 3.90 (s, 3H, OCH_3), 3.72 (m, 2H, H_k), 3.46–3.79 (m, 6H, H_a, H_b, H_m), 1.64 (m, 2H, H_l), 1.60–1.67 (m, 6H, H_c and H_d); ^{13}C NMR (DMSO-d_6) δ 115.6 (C_1), 154.0 (C_2), 125.06 (C_3), 106.34 (C_4), 117.55 (C_5), 135.66 (C_6), 122.73 (C_7), 127.07 (C_8), 177.51 (C_9), 136.32 ($C_{4'}$), 140.81 ($C_{10'}$), 120.25 ($C_{8'}$), 121.53 ($C_{9'}$), 56.26 (OCH_3), 49.58 (C_k), 22.83 (C_l), 43.93 (C_m), 48.84 (C_a and C_b), 9.54 (C_c and C_d). MS (*m*/*z*) 338 ($M^{+\cdot}$, 10), 337 (12), 86 (100), 72 (22), 36 (12).

10-[3′-[*N*-Bis(hydroxyethyl)amino]propyl]-2-methoxyacridone (4). Amounts of 1.16 g (3.84 mmol) of **2**, 1.64 g of KI, 2.65 g of K_2CO_3 and 1.20 mL (1.28 g, 9.83 mmol) of *N*,*N*-diethanolamine were refluxed for 24 h and followed the rest of the procedure used for **3**. The product was purified by column chromatography to give yellow oily product and it was converted into hydrochloride salt, which was dried over high vacuum to get pure solid (**4**) (0.93g, 45%, mp 192–193 °C). UV λ_{max} (∈) (MeOH) 212 (8177), 252 (32,838), 270 (30,211), 397 (6271) nm. IR 3379, 2970, 2867, 2779, 1636, 1594, 1501, 1480, 1434, 1274, 1258, 1227, 1129, 1196, 829, 767 cm^{-1}. ^1H NMR (DMSO-d_6) δ 7.70–8.77 (m, 7H, Ar-H, H_1, H_3, H_4, H_5-H_8), 4.17 (s, 2H, H_e and H_f), 3.93 (s, 3H, OCH_3), 3.64–3.88 (m, 8H, H_k, H_m, H_c, H_d), 2.50–2.75 (m, 4H, H_a and H_b), 1.40–1.60 (m, 2H, H_l); ^{13}C NMR (DMSO-d_6) δ 114.04 (C_1), 150.09 (C_2), 121.16 (C_3), 113.67 (C_4), 116.00 (C_5), 132.75 (C_6), 118.85 (C_7), 123.14 (C_8), 173.58 (C_9), 132.39 ($C_{4'}$), 136.88 ($C_{10'}$), 116.29 ($C_{8'}$), 117.34 ($C_{9'}$), 56.44 (OCH_3), 47.74 (C_k), 18.47 (C_l), 40.01 (C_m), 52.37 (C_a and C_b), 52.70 (C_c and C_d). MS *m*/*z* (%) 371 $(M+H)^+$, 100), 226 (30), 146 (21). Anal. ($C_{21}H_{26}N_2O_4$) C, H, N.

10-(3′-*N*-Pyrrolidinopropyl)-2-methoxyacridone (5). The procedure used for **4** was repeated with 1.05 g (3.33 mmol) of **2**, 1.48 g of KI, 2.4 g of K_2CO_3 and 1 g (14.08 mmol, 1.2 mL) of pyrrolidine. The residue was purified by column chromatography, and the oil was converted into hydrochloride salt of **5** (0.72 g, 41%, mp 156–158 °C). UV λ_{max} (∈) (MeOH) 214 (4050), 252 (22,688), 272 (19,103), 400 (4587) nm. IR 3415, 2971, 2684, 1646, 1596, 1560, 1501, 1146, 1134, 1072, 876, 749 cm^{-1}. ^1H NMR (DMSO-d_6) δ 7.3–8.4 (m, 7H, Ar-H, H_1, H_3, H_4, H_5-H_8), 3.89 (s, 3H, OCH_3), 3.86 (m, 2H, H_k), 2.99–3.54 (m, 6H, H_a, H_b, H_m), 1.86–2.35 (m, 6H, H_l, H_c and H_d); ^{13}C NMR (DMSO-d_6) δ 115.74 (C_1), 154.16 (C_2), 125.24 (C_3), 106.43 (C_4), 117.72 (C_5), 135.7 (C_6), 122.79 (C_7), 127.19 (C_8), 177.92 (C_9), 136.51 ($C_{4'}$), 140.96 ($C_{10'}$), 120.45 ($C_{8'}$), 121.74 ($C_{9'}$), 56.35 (OCH_3), 52.76 (C_k), 24.80 (C_l), 43.85 (C_m), 55.52 (C_a and C_b), 23.92 (C_c and C_d). MS *m*/*z* (%) 337 $(M+H)^+$, 100), 338 (22).

10-(3′-*N*-Piperidinopropyl)-2-methoxyacridone (6). The procedure used for **4** was repeated with 1.08 g (3.58 mmol) of **2**, 1.58 g of KI, 2.47 g of K_2CO_3 and 1.25 g (14.7 mmol, 1.45 mL) of piperidine. The crude product was purified by column chromatography to give a yellow oily product which was converted into hydrochloride salt of **6** (0.88 g, 44%, mp 220 °C). UV λ_{max} (∈) (MeOH) 214 (8217), 252 (33,062), 271 (27,751), 399 (6589) nm. IR 3405, 2944, 2692, 1625, 1599, 1568, 1512, 1475, 1294, 1284, 1180, 1067, 761, 697 cm^{-1}. ^1H NMR (DMSO-d_6) δ 7.6–8.75 (m, 7H, Ar-H, H_1, H_3, H_4, H_5-H_8), 3.84 (s, 3H, OCH_3), 3.20–3.75 (m, 4H, H_k and H_m), 2.90–2.95 (m, 4H, H_a and H_b), 1.82–2.23 (m, 8H, H_l, H_c, H_d and H_e); ^{13}C NMR (DMSO-d_6) δ 115.59 (C_1), 153.9 (C_2), 124.9 (C_3), 106.31 (C_4), 117.53 (C_5), 135.57 (C_6), 122.62 (C_7), 127.02 (C_8), 177.61 (C_9), 136.27 ($C_{4'}$), 140.78 ($C_{10'}$), 120.23 ($C_{8'}$), 121.5 ($C_{9'}$), 56.22 (OCH_3), 22.85 (C_l), 22.32 (C_e), 44.06 (C_m), 54.66 (C_a, C_b and C_k), 23.92 (C_c and C_d). MS *m*/*z* (%) ($M^{+\cdot}$, 10), 349 (15), 124 (20), 98 (100). Anal. ($C_{22}H_{26}N_2O_2$) C, H, N.

10-(3′-*N*-Morpholinopropyl)-2-methoxyacridone (7). The experimental procedure used for **4** is applicable with 1.05 g (3.48 mmol) of **2**, 1.48 g of KI, 2.4 g of K_2CO_3 and 1.12 g (12.8 mmol) of morpholine. The oily product was chromatographed on the silica gel to get pure product **7** and it was treated with ethereal hydrochloride to give hydrochloride salt of **7** (0.80 g, 42%, mp 212–213 °C). UV λ_{max} (∈) (MeOH) 209 (17,159), 252 (30,350), 270 (25,136), 398 (5486) nm. IR 3421, 2910, 2735, 1641, 1599, 1506, 1424, 1144, 1108, 1088, 946, 782, 709 cm^{-1}. ^1H NMR (DMSO-d_6) δ 7.73–8.78 (m, 7H, Ar-H, H_1, H_3, H_4, H_5-H_8), 3.71–3.84 (m, 7H, H_c, H_d and OCH_3), 3.46 (m, 4H, H_k and H_m), 2.68 (t, 4H, H_a and H_b), 1.40–1.51 (m, 2H, H_l); ^{13}C NMR (DMSO-d_6) δ 115.7 (C_1), 154.08 (C_2), 125.14 (C_3), 106.39 (C_4), 117.66 (C_5), 135.7 (C_6), 122.77 (C_7), 127.12 (C_8), 177.78 (C_9), 136.43 ($C_{4'}$), 140.89 ($C_{10'}$), 120.34 ($C_{8'}$), 121.63 ($C_{9'}$), 56.32 (OCH_3), 54.98 (C_k), 22.57 (C_l), 43.83 (C_m), 53.08 (C_a and C_b), 65.05 (C_c and C_d). MS *m*/*z* (%) 353 $(M+H)^+$, 100.

10-(3′-*N*-Thiomorpholinopropyl)-2-methoxyacridone (8). The experimental steps used for **4** were repeated by taking 1.13 g (3.74 mmol) of **2**, 1.59 g of KI, 2.58 g of K_2CO_3 and 1.25 g (12.12 mmol) of thiomorpholine. The oily product was purified by column chromatography and the yellow oily product was converted into hydrochloride salt of **8** (0.85 g, 40%, mp 152 °C). UV λ_{max} (∈) (MeOH) 215 (9950), 252 (28,168), 271 (24,752), 399 (5630) nm. IR 3431, 2919, 2789, 1646, 1620, 1589, 1465, 1424, 1253, 1139, 1072, 999, 891 cm^{-1}. ^1H NMR (DMSO-d_6) δ 7.2–8.3 (m, 7H, Ar-H, H_1, H_3, H_4, H_5-H_8), 3.85 (s, 3H, OCH_3), 3.72 (m, 2H, H_k), 2.50–2.54 (m, 6H, H_a, H_b and H_m), 2.49 (m, 4H, H_c and H_d), 1.85 (m, 2H, H_l). ^{13}C NMR (DMSO-d_6) δ 117.68 (C_1), 154.29 (C_2), 124.57 (C_3), 105.19 (C_4), 119.58 (C_5), 133.31 (C_6), 121.3 (C_7), 126.21 (C_8), 176.46 (C_9), 136.14 ($C_{4'}$), 140.85 ($C_{10'}$), 119.95 ($C_{8'}$), 121.01 ($C_{9'}$), 55.71 (OCH_3), 53.35 (C_k), 24.05 (C_l), 42.71 (C_m), 53.21 (C_a and C_b), 26.44 (C_c and C_d). MS *m*/*z* (%) 369 $(M+H)^+$, (5), 247 (100), 226 (90), 162 (58).

10-(3′-*N*-[Methylpiperazino)propyl]-2-methoxyacridone (9). The procedure used for **4** was employed with 1 g (3.32 mmol) of **2**, 1.41 g of KI, 2.29 g of K_2CO_3 and 1.10 g (11.0 mmol, 1.22 mL) of 1-methylpiperazine. The oily residue was purified by column chromatography and it was treated with ethereal hydrochloride to give hydrochloride salt of **9** (0.82 g, 42%, mp 218 °C). UV λ_{max} (∈) (MeOH) 212 (4701), 251 (29,003), 270 (27,569), 396 (5816) nm. IR 3421, 2955, 2665, 2567, 1636, 1599, 1517, 1465, 1253, 1113, 1015, 968, 777 cm^{-1}. ^1H NMR (DMSO-d_6) δ 7.42–8.47 (m, 7H, Ar-H, H_1, H_3, H_4, H_5-H_8), 3.30–3.98 (m, 15H, H_a, H_b, H_c, H_d, H_k, H_m and OCH_3), 2.91 (s, 3H, H_e), 2.20 (m, 2H, H_l); ^{13}C NMR (DMSO-d_6) δ 116.5 (C_1), 154.68 (C_2), 123.85 (C_3), 106.94 (C_4), 118.44 (C_5), 135.93 (C_6), 123.11 (C_7), 127.53 (C_8), 178.38 (C_9), 137.15 ($C_{4'}$), 141.54 ($C_{10'}$), 120.94 ($C_{8'}$), 122.21 ($C_{9'}$), 55.84 (OCH_3), 44.16 (C_k), 23.09 (C_l), 42.49 (C_m), 50.09 (C_a and C_b), 51.45 (C_c and C_d), 27.58 (C_e). MS *m*/*z* (%) 365 ($M^{+\cdot}$, 20), 266 (5), 252 (12), 239 (20), 225 (10), 210 (12), 141 (10), 127 (35), 113 (90), 99 (100), 85 (80). Anal. ($C_{22}H_{27}N_3O_2$) C, H, N.

10-(3′-*N*-[(β-Hydroxyethyl)piperazino]propyl)-2-methoxyacridone (10). Amounts of 1.09 g (3.61 mmol) of **2,** 1.54 g of KI, 2.49 g of K_2CO_3 and 2.03 g of (17.5 mmol, 2.15 mL) of (β-hydroxyethyl)piperazine were refluxed and processed according to the procedure used for **3**. The free base was purified by column chromatography and treated with ethereal hydrochloride to give hydrochloride salt of **10** (1.2 g, 43%, mp 228–230 °C). UV λ_{max} (∈) (MeOH) 214 (15,600), 252 (46,800), 271 (39,266), 399 (9400) nm. IR 3436, 2937, 2690, 1641, 1599, 1517, 1470, 1289, 1056, 1024, 824, 761 cm^{-1}. ^1H NMR (DMSO-d_6) δ 7.63–8.8 (m, 7H, Ar-H, H_1, H_3, H_4, H_5-H_8), 4.29 (t, 1H, H_g), 3.75 (s, 3H, OCH_3), 3.00–3.92 (m, 4H, H_k and H_f) 2.36–3.00 (m, 12H, H_a, H_b, H_c, H_d, H_e and H_m), 1.54 (m, 2H, H_l); ^{13}C NMR (DMSO-d_6) δ 115.7 (C_1), 153.99 (C_2), 124.34 (C_3), 106.08 (C_4), 117.93 (C_5), 134.07 (C_6), 122.24 (C_7), 126.72 (C_8), 176.07 (C_9), 136.34 ($C_{4'}$), 141 ($C_{10'}$), 120.71 ($C_{8'}$), 121.08 ($C_{9'}$), δ 55.78 (OCH_3), 53.36 (C_k), 23.74 (C_l), 43.13 (C_m), 49.79 (C_a and C_b), 51.35 (C_c and C_d), 55.45 (C_e), 57.97 (C_f). MS *m*/*z* (%) 396 $(M+H)^+$, 100.

10-(4′-Chlorobutyl)-2-methoxyacridone (11). Compound **11** (1.24 g, 53%), in the pure form was prepared by following the procedure used for **2**, with 1 g (4.44 mmol) of

2-methoxyacridone and 1-bromo-4-chlorobutane (11.5 mmol). UV λ_{max} (∈) (MeOH) 213 (16,165), 253 (48,667), 271 (41,275), 399 (9813) nm. IR 3436, 3012, 2968, 1645, 1636, 1599, 1478, 1369, 1276, 1179, 1067, 816, 784, 776 cm^{-1}. ^1H NMR (DMSO-d_6) δ 7.22–8.35 (m, 7H, Ar-H, H_1, H_3, H_4, H_5-H_8), 3.85 (s, 3H, OCH_3), 3.70–3.75 (m, 2H, H_n), 3.62 (m, 2H, H_k), 2.01 (m, 2H, H_l), 1.89 (m, 2H, H_m); ^{13}C NMR (DMSO-d_6) δ 115.86 (C_1), 153.9 (C_2), 122.32 (C_3), 106.12 (C_4), 115.86 (C_5), 133.85 (C_6), 120.96 (C_7), 125.91 (C_8), 175.76 (C_9), 135.66 ($C_{4'}$), 140.42 ($C_{10'}$), 118.05 ($C_{8'}$), 120.65 ($C_{9'}$), 55.38 (OCH_3), 45.03 (C_k), 45.19 (C_n), 24.39 (C_l), 25.65 (C_m). MS m/z (%) 316 $(M+H)^+$, (10), 315 (100), 224 (30), 91 (40).

10-[4′-(*N*-Diethylamino)butyl] - 2 - methoxyacridone (12). The procedure used for **3** was followed with 1.2 g (3.8 mmol) of **11**, 1.57 g of KI, 2.62 g of K_2CO_3 and 1.3 g (17.8 mmol) of *N*,*N*-diethylamine. The oily residue was purified by column chromatography and the pure base was converted into hydrochloride salt of **12** (1.26 g, 56%, mp 166 °C). UV λ_{max} (∈) (MeOH) 213 (15,361), 252 (42,527), 271 (35,583), 399 (8416) nm. IR 3440, 2951, 2684, 1625, 1599, 1553, 1501, 1480, 1284, 1191, 1030, 849, 839, 756 cm^{-1}. ^1H NMR (DMSO-d_6) δ 6.86–7.78 (m, 7H, Ar-H, H_1, H_3, H_4, H_5-H_8), 3.71 (m, 2H, H_k), 3.55 (s, 3H, OCH_3), 2.87–3.08 (m, 6H, H_a, H_b, H_n), 1.38–1.62 (m, 4H, H_l and H_m), 1.15 (m, 6H, H_c and H_d); ^{13}C NMR (DMSO-d_6) δ 114.5 (C_1), 152.52 (C_2), 123.82 (C_3), 106.31 (C_4), 116.3 (C_5), 135.56 (C_6), 121.25 (C_7), 125.62 (C_8), 176.13 (C_9), 134.97 ($C_{4'}$), 139.41 ($C_{10'}$), 118.81 ($C_{8'}$), 119.99 ($C_{9'}$), 54.86 (OCH_3), 50.86 (C_k), 23.35 (C_l), 20.25 (C_m), 45.11 (C_n), 47.17 (C_a and C_b), 8.10 (C_c and C_d). MS m/z (%) 353 $(M+H)^+$, 100. Anal. ($C_{22}H_{28}N_2O_2$) C, H, N.

10-[4′-[*N*-Bis (hydroxyethyl)amino]butyl]-2-methoxyacridone (13). Amounts of 1.01 g (3.20 mmol) of **11**, 1.32 g of KI, 2.21 g K_2CO_3 and 1.2 mL (9.83 mmol) of bishydroxyethylamine were refluxed and processed according to the procedure used for **4**. The crude product was chromatographed on silica gel to get pure free base and it was converted into hydrochloride salt of **13** (1 g, 51%, mp 152 °C). UV λ_{max} (∈) (MeOH) 214 (11,432), 253 (37,050), 372 (31,320), 399 (7443) nm. IR 3410, 3074, 2883, 1625, 1599, 1558, 1501, 1470, 1279, 1263, 1186, 1077, 854, 823, 767 cm^{-1}. ^1H NMR (DMSO-d_6) δ 7.29–8.32 (m, 7H, Ar-H, H_1, H_3, H_4, H_5-H_8), 4.17 (s, 2H, H_e and H_f), 3.83 (s, 3H, OCH_3), 3.73 (m, 6H, H_k, H_c and H_d), 3.61 (m, 2H, H_n), 2.47 (m, 4H, H_a and H_b), 1.78–1.91 (m, 4H, H_l and H_m); ^{13}C NMR (DMSO-d_6) δ 114.47 (C_1), 152.54 (C_2), 123.78 (C_3), 104.66 (C_4), 116.28 (C_5), 134.1 (C_6), 121.26 (C_7), 125.66 (C_8), 176.07 (C_9), 134.97 ($C_{4'}$), 139.42 ($C_{10'}$), 118.83 ($C_{8'}$), 120.01 ($C_{9'}$), 54.92 (OCH_3), 53.11 (C_k), 23.34 (C_l), 19.97 (C_m), 45.23 (C_n), 54.75 (C_a and C_b), 55.23 (C_c and C_d). MS m/z (%) 385 $(M+H)^+$, 100.

10 - (4′- *N* - Pyrrolidinobutyl) - 2 - methoxyacridone (14). Compound **14** as its hydrochloride salt (0.96 g, 47%, mp 132 °C) was obtained by following the procedure of **4** with 1 g (3.19 mmol) of **11**, 1.31 g KI, 2.18 g K_2CO_3 and 1.26 g (17.74 mmol) of pyrrolidine. UV λ_{max} (∈) (MeOH) 214 (13,822), 252 (34,903), 271 (29,725), 399 (7354) nm. IR 3431, 2955, 2701, 1636, 1599, 1553, 1512, 1475, 1289, 1186, 1051, 849, 813, 767 cm^{-1}. ^1H NMR (DMSO-d_6) δ 7–7.85 (m, 7H, Ar-H, H_1, H_3, H_4, H_5-H_8), 3.59 (s, 3H, OCH_3), 3.80 (m, 2H, H_k), 3.51 (m, 2H, H_n), 2.89–2.99 (m, 4H, H_a and H_b) 1.87–2.02 (m, 4H, H_c and H_d), 1.44–1.61 (m, 4H, H_l and H_m); ^{13}C NMR (DMSO-d_6) δ 116.34 (C_1), 154.58 (C_2), 125.66 (C_3), 106.79 (C_4), 118.25 (C_5), 135.82 (C_6), 122.99 (C_7), 127.45 (C_8), 178.27 (C_9), 137.02 ($C_{4'}$), 141.41 ($C_{10'}$), 120.86 ($C_{8'}$), 122.12 ($C_{9'}$), 56.77 (OCH_3), 55.74 (C_k), 25.13 (C_l), 23.83 (C_m), 46.59 (C_n), 55.57 (C_a and C_b), 24.07 (C_c and C_d). MS m/z (%) 351 $(M+H)^+$, 100.

10-(4′-*N*-Piperidinobutyl)-2-methoxyacridone (15). The method employed for **4** was used with 1.08 g (3.42 mmol) of **11**, 1.41 g of KI, 2.36 g of K_2CO_3 and 1.4 g (16.44 mmol) of piperidine. The crude product was purified by column chromatography and it was converted into hydrochloride salt of **15** (1.2 g, 53%, mp 119–121 °C). UV λ_{max} (∈) (MeOH) 214 (8348), 253 (36,071), 271 (30,401), 399 (7455) nm. IR 3415, 2987, 2704, 1646, 1631, 1625, 1501, 1279, 1181, 1025, 839, 755 cm^{-1}. ^1H NMR (DMSO-d_6) δ 6.86–7.75 (m, 7H, Ar-H, H_1, H_3, H_4, H_5-H_8), 3.53 (s, 3H, OCH_3), 3.68 (t, 2H, H_k), 3.30 (t, 2H, H_n), 2.70–2.95 (m, 4H, H_a and H_b), 1.31–1.77 (m, 10H, H_l, H_m, H_c, H_d and H_e); ^{13}C NMR (DMSO-d_6) δ 112.27 (C_1), 150.35 (C_2), 121.6 (C_3), 102.44 (C_4), 114.09 (C_5), 131.88 (C_6), 119.04 (C_7), 123.45 (C_8), 174 (C_9), 132.78 ($C_{4'}$), 137.22 ($C_{10'}$), 116.65 ($C_{8'}$), 119.84 ($C_{9'}$), 53.68 (OCH_3), 18.77 (C_l), 18.11 (C_e), 21.15 (C_m), 42.92 (C_n), 50.83 (C_a and C_b), 52.68 (C_k), 20.46 (C_c and C_d). MS m/z (%) 365 $(M+H)^+$, 100).

10-(4′-*N*-Morpholinobutyl)-2-methoxyacridone (16). The procedure used for **4** was repeated with 1.19 g (3.77 mmol) of **11**, 1.55 g of KI, 2.59 g of K_2CO_3 and 1.05 g (1.06 mL, 12.14 mmol) of morpholine. The product was purified by column chromatography and converted into hydrochloride salt of **16** (1.1 g, 53%, mp 206 °C). UV λ_{max} (∈) (MeOH) 214 (18,622), 253 (37,097), 272 (30,932), 399 (7521) nm. IR 3436, 2963, 2649, 1645, 1636, 1506, 1487, 1274, 1210, 1183, 1065, 942, 839, 727 cm^{-1}. ^1H NMR (DMSO-d_6) δ 6.89–7.81 (m, 7H, Ar-H, H_1, H_3, H_4, H_5-H_8), 4.00 (m, 2H, H_k), 3.70 (m, 4H, H_c and H_d), 3.56 (s, 3H, OCH_3), 3.38 (m, 2H, H_n), 3.05 (m, 4H, H_a and H_b), 1.41–1.65 (m, 4H, H_l and H_m); ^{13}C NMR (DMSO-d_6) δ 114.47 (C_1), 152.5 (C_2), 123.85 (C_3), 104.53 (C_4), 116.27 (C_5), 134.13 (C_6), 121.28 (C_7), 125.59 (C_8), 175.8 (C_9), 134.91 ($C_{4'}$), 139.33 ($C_{10'}$), 118.65 ($C_{8'}$), 119.84 ($C_{9'}$), δ56.27 (OCH_3), 54.90 (C_k), 23.22 (C_l), 20.02 (C_m), 45.27 (C_n), 51.58 (C_a and C_b), 63.68 (C_c and C_d). MS m/z (%) 367 $(M+H)^+$, 100. Anal. ($C_{22}H_{26}N_2O_3$).

10-(4′-*N*-Thiomorpholinobutyl)-2-methoxyacridone (17). Amounts of 1.11 g (3.52 mmol) of **11**, 1.45 g of KI, 2.42 g K_2CO_3 and 1.3 g (12.6 mmol) of thiomorpholine were refluxed and processed according to the procedure used for **4**. The crude product was purified by column chromatography and the light-yellow oily product was converted into hydrochloride salt of **17** (1 g, 49%, mp 163 °C). UV λ_{max} (∈) (MeOH) 214 (13,189), 255 (35,412), 271 (30,689), 399 (7564) nm. IR 3426, 2965, 2872, 1636,

1609, 1599, 1512, 1465, 1361, 1289, 1175, 1030, 945, 836, 761 cm^{-1}. ^1H NMR (DMSO-d_6) δ 7.2–8.3 (m, 7H, Ar-H, H_1, H_3, H_4, H_5–H_8), 3.83 (s, 3H, OCH_3), 3.76 (m, 2H, H_k), 2.52–2.56 (m, 6H, H_a, H_b and H_n), 2.43 (m, 4H, H_c and H_d), 1.85–2.00 (m, 4H, H_l and H_m); ^{13}C NMR (DMSO-d_6) δ 114.74 (C_1), 151.69 (C_2), 122.98 (C_3), 106.11 (C_4), 116.27 (C_5), 134.27 (C_6), 121.7 (C_7), 125.87 (C_8), 177.12 (C_9), 134.89 ($C_{4'}$), 139.33 ($C_{10'}$), 118.62 ($C_{8'}$), 119.74 ($C_{9'}$), 55.73 (OCH_3), 53.30 (C_k), 23.22 (C_l), 20.21 (C_m), 42.77 (C_n), 53.22 (C_a and C_b), 26.38 (C_c and C_d). MS m/z (%) 383 $(M+H)^+$, 10), 224 (100).

10-(4′-*N*-(Methylpiperazino)butyl)-2-methoxyacridone (18). The procedure used for **3** was followed with 1.09 g (3.45 mmol) of **11**, 1.43 g of KI, 2.38 g of K_2CO_3 and 1.56 g (15.6 mmol) of 1-methylpiperazine. The crude product was purified by column chromatography and the oily product was treated with ethereal hydrochloride to get hydrochloride salt of **18** (1.4 g, 57%, mp 223–224 °C). UV λ_{max} (∈) (MeOH) 209 (27,923), 253 (32,140), 271 (25,814), 399 (5846) nm. IR 3436, 2965, 2761, 1634, 1537, 1501, 1478, 1287, 1174, 1031, 847, 767 cm^{-1}. ^1H NMR (DMSO-d_6) δ 7.18–8.08 (m, 7H, Ar-H, H_1, H_3, H_4, H_5-H_8), 3.73 (s, 3H, OCH_3), 3.25–3.56 (m, 12H, H_a, H_b, H_c, H_d, H_k and H_n), 2.94 (s, 3H, H_e), 1.70–1.79 (m, 4H, H_l and H_m,); ^{13}C NMR (DMSO-d_6) δ 116.5 (C_1), 154.68 (C_2), 125.77 (C_3), 106.94 (C_4), 118.44 (C_5), 135.93 (C_6), 123.11 (C_7), 127.53 (C_8), 178.38 (C_9), 137.15 ($C_{4'}$), 141.54 ($C_{10'}$), 120.94 ($C_{8'}$), 122.21 ($C_{9'}$), 57.85 (OCH_3), 56.90 (C_k), 25.13 (C_l), 22.05 (C_m), 46.53 (C_n), 50.11 (C_a and C_b), 51.69 (C_c and C_d) 44.45 (C_e). MS m/z (%) 380 $(M+H)^+$, 100).

10-[4′-*N*-(β-Hydroxyethyl)piperazino)butyl]-2-methoxyacridone (19). Amounts of 1.2 g (3.80 mmol) of **11**, 1.57 g of KI, 2.62 g of K_2CO_3 and 2.2 g (2.1 mL, 16.9 mmol) of (β-hydroxyethyl)piperazine were refluxed and processed according to the experimental conditions for **4**. The free base was purified by column chromatography, a pure yellow solid of **19** was obtained (1.3 g, 43%, mp 136 °C). UV λ_{max} (∈) (MeOH) 213 (21,410), 252 (51,534), 272 (43,360), 399 (10,569) nm. IR 3379, 2953, 2676, 1642, 1594, 1506, 1491, 1274, 1174, 1067, 863, 767 cm^{-1}. ^1H NMR (DMSO-d_6) δ 7.7–8.77 (m, 7H, Ar-H, H_1, H_3, H_4, H_5–H_8), 4.77 (t, 1H, H_g), 3.73 (s, 3H, OCH_3), 3.92 (m, 2H, H_f), 3.71 (m, 2H, H_k), 2.49–3.00 (m, 12H, H_a, H_b, H_c, H_d, H_e and H_n), 2.08–2.25 (m, 4H, H_l and H_m); ^{13}C NMR (DMSO-d_6) δ 116.14 (C_1), 154.2 (C_2), 124.41 (C_3), 106.35 (C_4), 118.32 (C_5), 134.1 (C_6), 122.65 (C_7), 127.01 (C_8), 176.12 (C_9), 136.6 ($C_{4'}$), 141.29 ($C_{10'}$), 121.12 ($C_{8'}$), 121.12 ($C_{9'}$), 58.89 (OCH_3), 55.73 (C_k), 24.40 (C_l), 22.80 (C_m), 45.39 (C_n), 53.10 (C_a and C_b), 53.73 (C_c and C_d), 56.67 (C_e), 60.79 (C_f). MS m/z (%) 410 $(M+H)^+$, 100. Anal. ($C_{24}H_{31}N_3O_3$) C, H, N.

Biological activity

Determination of pK_a and partition coefficient. The pK_as were determined according to a previously published method.[31] Briefly, 10 mL of an approximately 10^{-3} M solution of each compound was titrated with 1 M hydrochloric acid or sodium hydroxide using a combination glass electrode to measure pH. The relative lipophilicity was assessed for each of the derivatives using an adaptation of the method of Zamora et al.[32] This method involves measuring the partitioning of the compound between 1-octanol and 0.1 M PBS buffer (pH 7.4). HPLC-grade 1-octanol was presaturated with PBS, and aqueous phase PBS was saturated with 1-octanol before use. The derivatives were each dissolved in 1-octanol/buffer phase at final concentration of 10^{-4} M, and an equal volume of buffer/1-octanol was added. The tubes were then inverted continuously for 15 min; control experiments confirmed that equilibrium was reached within this time. The final concentration of compound in both aqueous and octanol phases was measured by UV spectrophotometry. The partition coefficient, P, was determined by dividing the concentration of the derivative in 1-octanol by the concentration in the aqueous phase.

Cell lines and cell culture. Human epidermoid carcinoma KB-3-1 cells and a colchicine-selected MDR variant, $KBCh^R$-8-5 cells cross-resistant to vincristine (45-fold) and VLB (~10-fold) and KB-V1 cells were grown in monolayer culture in antibiotic-free Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum and L-glutamine in a humidified atmosphere of 5% CO_2 in air. The resistance of $KBCh^R$-8-5 and KB-V1 cells was maintained by culturing them respectively with 10 ng/mL of colchicine and 1 ng/mL of VLB.

Accumulation studies. Cells (2×10^6 cells) were plated and incubated overnight at 37 °C to attach to plastic. Medium was aspirated and cells were washed with 2×2 mL PT buffer (120 mM NaCl, 20 mM Tris-base, 3 mM K_2HPO_4, 10 mM glucose, 0.5 mM $MgCl_2$ and 1 mM $CaCl_2$, pH 7.4). Monolayers were incubated at room temperature for 10 min in PT buffer prior to aspiration and adding 1 mL of serum-free RPMI-1640HEPES buffer (10.4 g RPMI-1640 medium in 1 L of 25 mM HEPES, pH 7.4) containing 49.9 nM [^{3}H] VLB with or without VRP or 2-methoxy-N^{10}-substituted acridones (**1**–**19**) at 100 μM dissolved in DMSO (final culture concentration <0.1% DMSO). After 2 h of incubation at room temperature, medium was rapidly aspirated to terminate the drug accumulation and monolayers were washed four times with ice-cold PBS (g/L:NaCl 8.0; $Na_2HPO_4{\cdot}12H_2O$ 2.9; KCl 0.2; KH_2PO_4 0.2) buffer and drained. To each dish 1 mL of trypsin-EDTA (0.05% trypsin, 0.53 mM EDTA) was added. After 1 min, monolayers were triturated to give a uniform suspension of cells and radioactivity in 0.75 mL was determined by scintillation counting. Cell number per dish was determined using hemocytometer and amount of intracellular VLB was determined (Table 1).

Measurement of VLB efflux. Cells (2×10^6/dish) were plated and incubated overnight at 37 °C in a CO_2 incubator to attach to plastic. Medium was removed and monolayer cells were washed once with 3 mL of PT

buffer and incubated for 10 min in another 2 mL of the same buffer. KBCh^R-8-5 cells were incubated with 1 mL of serum-free RPMI-1640HEPES buffer, pH 7.4 containing 49.9 nM [^{3}H] VLB for 2 h at room temperature. Drug solutions were aspirated and 1 mL of the same buffer without or with modulators (**1**–**19**) or VRP at 100 μM were added and incubated for 2 h more at room temperature. The medium was aspirated from each dish and the cells were washed four times in ice-cold PBS and drained. Cells were harvested and radioactivity per dish was calculated as described above and the results are given in Table 2.

Competition for [^{3}H] azidopine labeling of P-glycoprotein. Preparation of plasma membrane fractions and photoaffinity labeling. Competition assay for photolabeling of P-gp used membranes from KB-V1 cells, which have higher P-gp levels than do KBCh^R-8-5 cells. Crude membranes were prepared from the MDR variant, KB-V1, essentially as described previously.[33]

Briefly $>10^9$ KB-V1 cells were harvested, washed several times in 25 mM HEPES, 130 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 10 mM glucose, 5 mM NaH_2PO_4, pH 7.4, finally resuspended at 10^7 cells/mL in 20 mM HEPES, 150 mM NaCl, 2 mM $MgCl_2$, 1 mM EDTA, 1 mM phenylmethylsulfonyfluoride, pH 7.4 and lysed by nitrogen cavitation; membrane pellets were suspended in 100 mM Tris, pH 7.4 and frozen at −70 °C until use. For photoaffinity labeling, membrane protein (200 mg) in buffer containing 250 mM sucrose and 10 mM Tris·HCl, pH 7.4 at 25 °C, was mixed with 100 nM [^{3}H] azidopine (50 Ci/mmol; Amersham) in the absence or presence of 100 mM modulators (**1**–**19**), in a total volume of 150 μL. After incubating for 20 min in the dark, the mixture was then irradiated with a germicidal UV-light (30 W), commonly used in laminar flow hoods, for 20 min at a distance of 10 cm.

SDS-PAGE and autoradiography. Photolabeled proteins were separated by one-dimensional 5–15% SDS-PAGE under reducing conditions using the discontinuous buffer system of Laemmli.[34] After staining with Coomassie Blue and destaining, the gels were soaked in Amplify (Amersham Corp.) for 30 min and dried under vacuum at 75 °C. The dried gels were exposed to film for 2 to 3 days at −70 °C and developed. Radioactively labeled bands were scanned in their centres with a densitometer and quantitated by integration with a recorder.

Inhibition of cellular proliferation. KB-3-1 or KBCh^R-8-5 at 5000 cells per well were plated in triplicate in 6-well flat-bottom tissue culture plates (Greiner GmbH, Germany). After 24 h at 37 °C in an atmosphere of 5% CO_2/95% air, medium was replaced with 3 mL of fresh medium containing acridone compounds (**1**–**19**) at concentration ranging from 0–100 μM in DMSO (final culture concentration <0.1%, DMSO) and cells were incubated at 37 °C for a further 7 days. The medium was aspirated and cells were washed once with 2 mL of 0.9% saline and dried overnight. Colonies were stained with 1 mL of 0.1% crystal violet followed by washing twice with distilled water and dried overnight. The IC_{10} and IC_{50} values (Table 3) were determined from concentration percent cell survival curves and were defined as the concentrations required for 10 and 50% reduction in colonies compared to controls.

Effect of 2-methoxy-N^{10}-substituted acridones on in vitro cytotoxicity of VLB. To determine the effects of the modulators on the cytotoxicity of VLB, KBCh^R-8–5 cells were treated with serial dilutions of VLB (0.0–100 nM) in the absence or presence of IC_{10} concentration of modulators. After incubation for 7 days at 37 °C, colonies were enumerated as described above. IC_{50} values were determined as previously described and the fold-potentiation (Table 4) was calculated from dividing the IC_{50} for VLB in the absence of modulator by the IC_{50} in the presence of modulating agent.

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