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Modulation of Human Mammary Cell Sensitivity to Paclitaxel by New Quinoline Sulfonamides

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Abstract—Sulfonamide derivatives of chloroquine and primaquine were synthesised and evaluated against both paclitaxel-sensitive and paclitaxel-resistant mammarian cancer cell lines. All derivatives exhibited at least 96% MDR reversal activity when co-administered with paclitaxel at 5 μM. The best compound, a chloroquine derivative, exhibited 99% MDR reversal activity when co-administered with paclitaxel at 1 μM. Molecular modelling studies reveal that these derivatives share a common pharmacophore with taxane MDR reversal agents. © 2001 Elsevier Science Ltd. All rights reserved.

One major problem in cancer chemotherapy is cellular resistance to structurally and functionally unrelated chemotherapeutic agents. This multidrug resistance (MDR) phenotype is often associated with an increase in P-glycoprotein on the cell surface.[1] P-glycoprotein, a 170-kDa plasma membrane protein, acts as an energy-dependent drug efflux pump and its overexpression results in reduced cellular drug accumulation. In the absence of a 3-D structure, the design of MDR reversal (MDRR) agents has relied on previous structure–activity relationship (SAR) studies. Although there has been several of these, most have pointed to the importance of a hydrophobic, conjugated planar ring as a key structural requirement.[2]

Paclitaxel (Taxol™) **1** is one of the best anticancer agents to be discovered in recent years.[3] In 1992, paclitaxel was approved by the US FDA for the treatment of advanced ovarian cancer and in 1994 for breast cancer. Its clinical use has been expanded to the treatment of lung, head, neck and gastrointestinal cancers. Paclitaxel has a novel mode of action in promoting tubulin assembly and stabilising the resulting microtubules.[4,5] Unfortunately, like with many anticancer drugs, resistance to paclitaxel has presented new challenges. Thus restoring paclitaxel sensitivity to resistant tumours is of vital importance.

The ability of the antimalarial drugs chloroquine **2** and primaquine **3** as well as other quinolines to act as MDRR agents for vinblastine-resistant (CEM/VLB100) and vincristine-resistant (K562/ADM) cancer cell lines has been previously reported.[6–8] Within the context of paclitaxel chemosensitising agents, some of the quinidine, quinine and quinacrine antimalarial drugs have recently been reported to overcome in vitro and in vivo paclitaxel resistance in vinblastine-resistant (CEM/VLB100), non-Hodgkin's lymphoma and hormone-refractory prostate cancer cells.[9–11] In this paper, we report on the preliminary design, synthesis and biological evaluation of new quinoline sulfonamides **4** and **5** with activity as paclitaxel chemosensitising agents (Fig. 1).

Since the discovery of the chemosensitisation of MDR cancer cell lines by **2** and **3** in the 1980s, little work has been done to exploit these antimalarial agents as MDRR agents in cancer. Among other things, we are interested in exploring possible similarities in the mechanisms of quinoline resistance in malaria and mammalian MDR in cancer vis-à-vis the involvement of P-glycoprotein in the former. Following on from the

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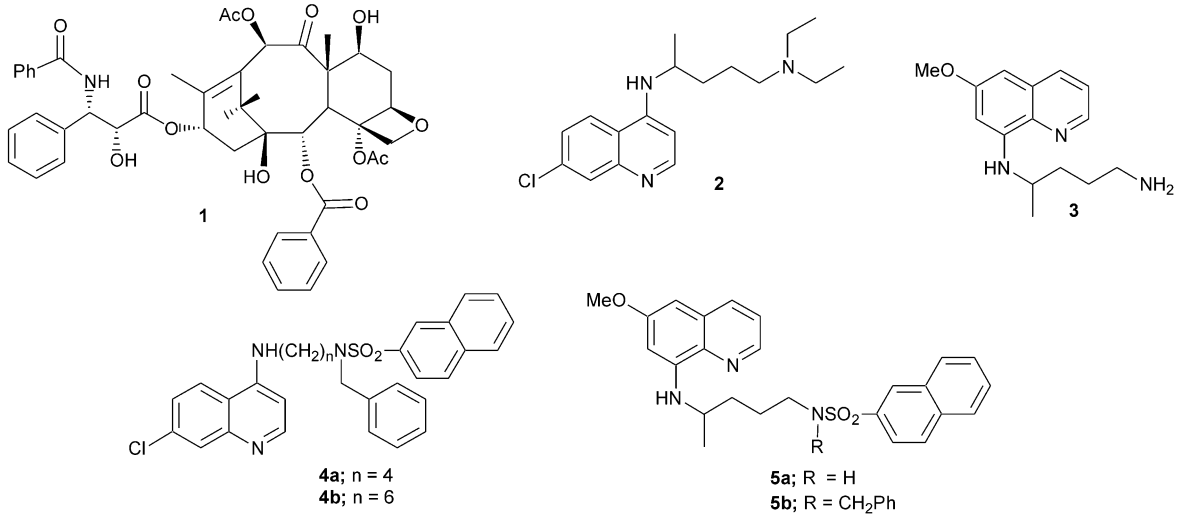


Figure 1. Structures of paclitaxel **1**, chloroquine **2**, primaquine **3**, and sulfonamides **4** and **5**.

previously described amide-based 5-alkoxyquinolines with MDRR activity,[8] we targeted some aromatic sulfonamide derivatives with varying methylene spacer lengths for preliminary studies. This is in view of recent observations that chloroquine derivatives with appropriate alkyl chain spacer lengths appear to evade the mechanism(s) responsible for resistance to **2** in malarial cells.[12–14] Since hydrophobicity is an important feature in MDRR agents, we reasoned that the presence of a sulfonamide proton should provide us with a diversity point for introducing various hydrophobic groups during the exploration of SARs.

The synthesis of derivatives **4a** and **5b** was trivial and straightforward and is exemplified in Scheme 1. Primaquine derivatives **5a** and **5b** were synthesised in a similar manner from the free base **3**. All new compounds gave ^{1}H NMR, FABMS and, in relevant cases, microanalysis data consistent with their structures.

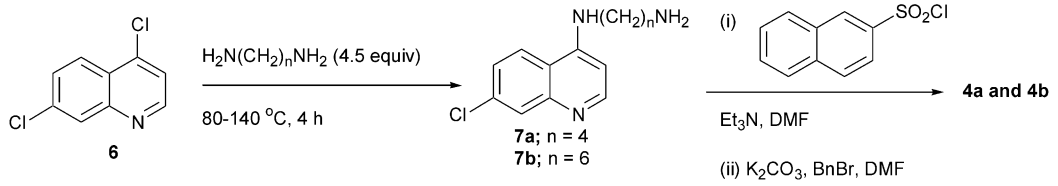
In order to establish the intrinsic anticancer activity, derivatives **4** and **5** were first tested against paclitaxel-sensitive (LCC-WT-human breast carcinoma) and paclitaxel-resistant (LCC6-MDR-MDR1 transfected) cell lines, respectively.[15] Cells were seeded at 2000 cells/well in 96-well plates in complete medium-RPMI-1640 containing 5% FCS, 5% Nuserum IV, 2 mM L-glutamine and 10 mM HEPES. Following overnight incubation, compounds were solubilized in 100% DMSO, diluted in complete medium and added to cell plates. After 72 h, cells were fixed, stained and total protein/well was determined. Compound concentration that inhibited growth by 50% was determined and reported as IC_{50}. The data are summarised in Table 1. The compounds generally showed no or very weak cytotoxicity at a test concentration of 10 μM in both cell lines. The only exception was compound **4b**, which was found to be cytotoxic in both cell lines.

The compounds were then co-administered with paclitaxel at four different concentrations as shown in Table 2. Paclitaxel recovered 96–99% of its efficacy against the resistant human breast cancer cells when compounds **4** and **5** were co-administered at a concentration of 5 μM. However, when co-administered with paclitaxel at a concentration of 1 μM, only compound **4b** exhibited 99% MDR reversal activity. Based on this, and the result with compound **4a** in comparison with the results from compounds **5**, it is apparent that the chloroquine series **4** is superior to the primaquine series **5**. It is noteworthy that primaquine compounds **5a** and **5b** are racemates and the biological activity may reside in one enantiomer only. As such, higher potency may be expected from the appropriate single enantiomer.

Table 1. Cytotoxicity of quinolines **4** and **5** against sensitive and resistant human breast cancer cells[a]

Compound	IC_{50} (μM)±SD		% Growth inhibition at 10 μM	
	LCC6-WT	LCC6-MDR	LCC6-WT	LCC6-MDR
Paclitaxel **1**	0.008±0.001	0.612±0.021		
4a	>10	>10	6	3
4b	1.7±0.09	2.0±0.24		
5a	>10	>10	16	8
5b	>10	>10	0	8

[a]LCC6-WT-human breast carcinoma; LCC-MDR-MDR1 transfected line.



Scheme 1. Synthesis of target compounds **4a** and **4b**.

Table 2. Modulation of human mammary cell sensitivity to paclitaxel by quinolines **4** and **5**

Compound	Concentration (μM)[a]	IC_{50} (nM) LCC6-MDR	% IC_{50} reduction
Paclitaxel		490	0
Paclitaxel + (**5a**)	5	13	97
Paclitaxel + (**5b**)	5	19	96
Paclitaxel + (**4a**)	5	2.7	99
Paclitaxel + (**4b**)	nd[b]	nd	nd
Paclitaxel + (**5a**)	1	353	36
Paclitaxel + (**5b**)	1	249	55
Paclitaxel + (**4a**)	1	164	70
Paclitaxel + (**4b**)	1	6.2	99
Paclitaxel + (**4b**)	0.3	254	54
Paclitaxel + (**4b**)	0.1	338	39

[a]No growth inhibition was seen at this concentration of reversal agent.
[b]Not determined due to cytotoxicity at 5 μM.

Since antimalarial drug analogues, **4** and **5**, exhibited substantial MDRR activity against LCC6-ADR when co-administered with paclitaxel, we thought there might be a common pharmacophore for these sulfonamide-based compounds and taxane-based MDRR agents that were developed earlier. Accordingly, we selected **4a** and **4b** as well as taxane MDRR agents (tRAs), SB-RA-30001 and SB-RA-31012,[16] and photoaffinity labelling taxoid {^{3}H}-SB-T-5111[17] for molecular modelling study (Fig. 2), and investigated common structural features between these two totally different classes of compounds.

The molecular modelling was performed on a Silicon Graphics O_2 workstation using two programs: SYBYL 6.4 and InsightII 2000. The quinolinesulfonamides structures, **4a** and **4b**, were constructed in SYBYL 6.4 and energy-minimised (Tripos force field, charges calculated by the Gasteiger–Huckel method). The lowest energy conformations of SB-RA-30001 and SB-RA-31012, and photoaffinity taxoid {^{3}H}-SB-T-5111 were obtained using SYBYL 6.4 by modifying the X-ray structure of paclitaxel and minimising the resultant conformers. The template forcing was performed using the InsightII 2000/Discover Module (tRAs or the taxoid as the template and **4a** or **4b** as the mover) using constant valence force field (CVFF). The resultant conformers of **4a** and **4b** were energy-minimised so that the energies of these conformers were within the 5 kcal/mol range from that of the lowest energy conformation. Then, these minimised conformers of **4a** and **4b** were overlaid with the tRAs and the taxoid. The overlay results are shown in Figure 3.

As Figure 3a shows, the two naphthyl moieties of **4b** and the phenyl group of the C-2 benzoate as well as the naphthalene group of the C-7 acyl moiety of SB-RA-30001 have almost perfect fit. This is a rather surprising finding. SB-RA-30001 possesses a high MDRR activity with paclitaxel (97.5% at 1 μM against human breast cancer cell line MCF7-ADR).[16] As compared to **4b**, the

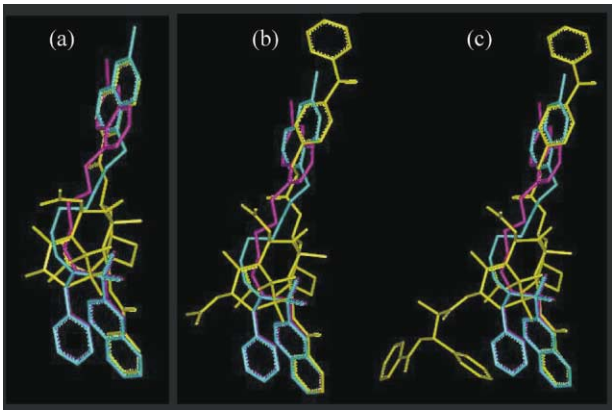


Figure 3. Molecular modelling graphics of quinolines overlaid with tRAs and photoaffinity labelling taxoid.

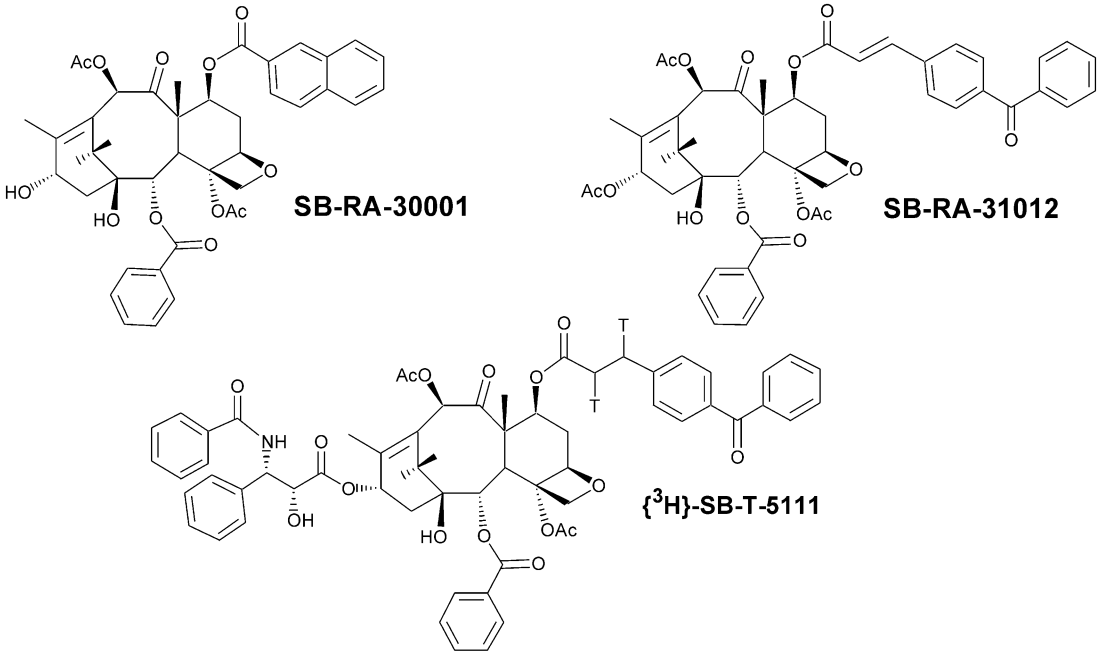


Figure 2. Structures of taxane MDRR agents (tRAs) SB-RA-30001 and SB-RA-31012 and photoaffinity labelling taxoid {^{3}H}-SB-T-5111.

overlap of the naphthalene moiety of **4a** with the naphthalene group of the C-7 acyl moiety of SB-RA-30001 is only partial. This observation is consistent with the difference in activity of **4a** and **4b**. Figure 3b shows the overlay of **4a**, **4b** and SB-RA-31012, that is a highly potent MDRR agent for paclitaxel (99% reversal activity at 1 μM and 92% at 0.1 μM against LCC6-ADR).[16] In this case, one of the aromatic rings of the naphthyl moiety of **4b** overlaps with one of the phenyl groups of the benzophenone moiety of SB-RA-31012, but **4a** does not have overlap in this part of the molecule. As Table 2 shows, **4b** exhibits high potency (99% reversal activity) at 1 μM, but the potency drops to 33% at 0.1 μM. This might be due to the fact that the methylene chain of **4b** is not long enough to reach a more preferable hydrophobic binding site to which the benzophenone moiety of SB-RA-31012 binds.

Figure 3c shows the overlay of **4a**, **4b** and {^{3}H}-SB-T-5111, that has been used for the photoaffinity labelling of Pgp.[17] As anticipated, the overlaps in the C-2 benzoate moiety and the tethered benzophenone moiety at C-7 with **4a** and **4b** are essentially the same as the case of SB-RA-31012. It should be noted that the phenyl group of the benzylamino moiety of **4a** and **4b** is located in the 'hydrophobic clustering' region of the taxoid. In addition, the phenyl and the naphthyl groups are apparently clustered through aromatic π–π interaction. As discussed above, the molecular modelling study has disclosed a probable common pharmacophore for these structurally very different classes of compounds, which provides a good rationale for the excellent MDRR activity of antimalarial drug related quinolinesulfonamides when used with paclitaxel.

In conclusion, we have shown the potential of quinoline-based compounds that chemosensitise cancer cells to paclitaxel. Like the previously described taxane-based paclitaxel chemosensitisers,[16,18] these compounds are practically non-cytotoxic themselves. As such they hold promise. Coupled with the simplicity of the chemistry, exploration of SAR studies and design of more potent and non-toxic compounds should be facilitated. Further investigation on the design, synthesis and SAR study of novel quinoline MDRR agents are actively underway in these laboratories.

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