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REGULAR ARTICLE

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The role of small molecule–small molecule interactions in overcoming biological barriers for antibacterial drug action

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Abstract The ineffectiveness of antibiotics against bacteria can be caused by multidrug resistance (MDR) or by an outer membrane, which restricts the penetration of amphipathic compounds into Gram-negative bacteria. Remarkable activities of plant antimicrobials in the presence of MDR modulators have been observed against a series of MDR and Gram-negative bacteria (Tegos et al., Antimicrob Agents Chemother 46:3133, 2002). Assuming that modulators of MDR might form complexes with substrates of efflux pumps Zloh et al., Biogr Med Chem Lett 14:881, 2004), we have evaluated interaction energies between antimicrobials and MDR modulators reported in Tegos et al. (Antimicrob Agents Chemother 46:3133, 2002). In this paper, we can confirm that modulation activity against the efflux pump NorA in *Staphylococcus aureus* correlates with the interaction energies between MDR modulator INF271 and antibacterials. Additionally, the change of log *P* of complexes might be responsible for overcoming the membrane impermeability in Gram-negative bacteria and increasing the antibacterial activity in the presence of the modulator MC207110. This suggests that interactions between small molecules may play an important role in overcoming biological barriers in bacteria.

Keywords Multidrug resistance · MDR · Antibiotic activity · MDR modulators · Intermolecular interactions · GRID

1 Introduction

Plants produce compounds that can be effective antimicrobials if they find their way into the cell of the pathogen [3]. Production of multidrug resistance (MDR) inhibitors by the plant would be one way to ensure delivery of the antimicrobial compound.

Gram-negative bacteria have an effective permeability barrier, comprised of the outer membrane, which restricts the penetration of amphipathic compounds. Additionally these bacteria possess multidrug resistance pumps (MDRs), which extrude toxins across this barrier. There is a direct assumption that the apparent ineffectiveness of plant antimicrobials is largely due to the permeability barrier. This hypothesis was tested in a study by Tegos and co-workers by evaluating a combination of MDR mutants and MDR inhibitors [1].

Several important results that have greatly raised our interest were observed in [1]. It was shown that the modulators INF271 and MC207110 significantly potentiated the actions of most antimicrobials in the panel against *Bacillus megaterium* and *Staphylococcus aureus*. The plant antimicrobials resveratrol and coumestrol showed little direct activity, and the MDR inhibitors caused little potentiation of activity by these antimicrobials. Gossypol presented an interesting anomaly: the activity of this plant antimicrobial decreased by approximately tenfold in the presence of INF_{271} in the mutant type, and a fourfold decrease in activity was observed in the MDR *norA* strain. Rhein, plumbagin, resveratrol, gossypol, coumestrol, pyrithione and berberine – all showed similar patterns of activity against Gram-negative species. All were relatively ineffective against most species in the panel, but the activity of each compound was strongly potentiated by MDR inhibitors against several of the species. This suggests that a high level of activity of an antimicrobial against a given species was achieved in the presence of modulators of MDRs responsible for efflux of the tested compound.

It is believed that MDR inhibitors often reverse multidrug resistance by competing for the transport system responsible for MDR, but overall the mechanism of MDR inhibition is not well understood. The relationships between drug structure and MDR, and inhibitor structure and MDR inhibition are still obscure, except for the molecule lipophilicity. MDR inhibitors may bind directly to the efflux protein, however, we have proposed that these inhibitors could also function

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as 'chaperones' by binding therapeutics outside the cell [2], increasing their lipophilicity (a key feature of all known MDR inhibitors) and therefore facilitating their entry into the cell. Furthermore, we have proposed that inhibitors of MDR have affinity for substrates of efflux transporters, and that they may form complexes. The experimental evidence has shown that indole-3-carbinol may have an affinity for substrates of P-glycoprotein (P-gp) and bind them to form a complex that may facilitate entry of the drug [4].The small molecule–small molecules interactions could play a key role in this large inhibitor–antibiotic complex formation and overcome biological barriers. Such a complex would effectively be a 'Trojan horse' that would not be recognised as a substrate by the pump mechanism. This complex may then dissociate to allow the inhibitor to bind to the pump causing inhibition of efflux and release of drug to its target.

We have used molecular modelling methods to rationalise results of the study by Tegos et al. Antimicrobials modelled were asarinin, esculetin, bisnorargemonine, 13-hydroxylupanine, coumestrol, rhein, plumbagin, pyrithione, resveratrol, gossypol, and berberine (Scheme 1). Bisnorargemonine and 13-hydroxylupanine were not discussed in detail, since they do not possess antibacterial activity. The interaction between antimicrobials and well-characterised MDR inhibitors INF271 and MC207110 (Scheme 2) was predicted by the Glue docking module of Grid22 [5]. Molecular properties of complexes with most favourable interaction energies were evaluated using VegaZZ software [6, 7]. The possibility of complex formation, correlation of antibacterial activity with interaction energies and increase of octanol/water partition coefficient, ($\log P$) of antibacterial-MDR inhibitor complex might provide a new insight into the mechanism of MDR modulation.

2 Computational methods

2.1 Model building and conformational analysis of antibacterials and MDR inhibitors

The initial structures of INF271, MC207110 and a series of antibacterials were sketched using ChemDraw Ultra 7.0.1, converted into three-dimensional (3D) models and saved as mol2 files by Chem3D Ultra 7.0.0 [8]. These structures were imported into Macromodel [9], atom and bond types were adjusted and minimised with the MMFFs force field parameters [10]. The generalised Born/surface area continuum (GB/SA) solvent model for H_2O [11] implemented in MacroModel was used to simulate an aqueous environment, with a constant dielectric function ($\varepsilon = 1$). An extended non-bonded cutoff (van der Waals: 8 Å; electrostatics: 20 Å) was used.

Using the optimised structures, a systematic conformational search on each molecule was performed. Monte Carlo conformational analysis (500 steps) was used for all compounds. The energy cutoff was set to $\Delta E = 10$ kJ/mol above the lowest energy conformation. The ensembles of generated structures were clustered using the program Xcluster. All structure sets were analysed using the cluster analysis program Xcluster [12].

2.2 Prediction of interaction energies between antibacterials and MDR inhibitors

Representative structures of inhibitors and drugs were used as input files for GRID 22 software [5]. The whole molecule was considered during calculations and all GRID parameters were kept at their default values. Glue, the GRID-docking programme, was used to find possible interactions for an antibacterial molecule with an inhibitor molecule as a target. All interactions were examined by using GROUP probes implemented in the Glue software representing relevant functional groups of drug molecules. Reported interaction energies by Glue 1.0 software were used as a criteria for evaluating the probability of MDR modulation. Complexes between antibacterials and modulators that exhibited the most favourable interaction energies were saved as mol2 files and imported into MacroModel. The complexes were further optimised using MMFFs force field.

The MINTA calculations of free energy [13] of single molecules and complexes were used to predict relative binding energies of different antibacterials to a given modulator by using a thermodynamic cycle.

2.3 Calculation of molecular properties

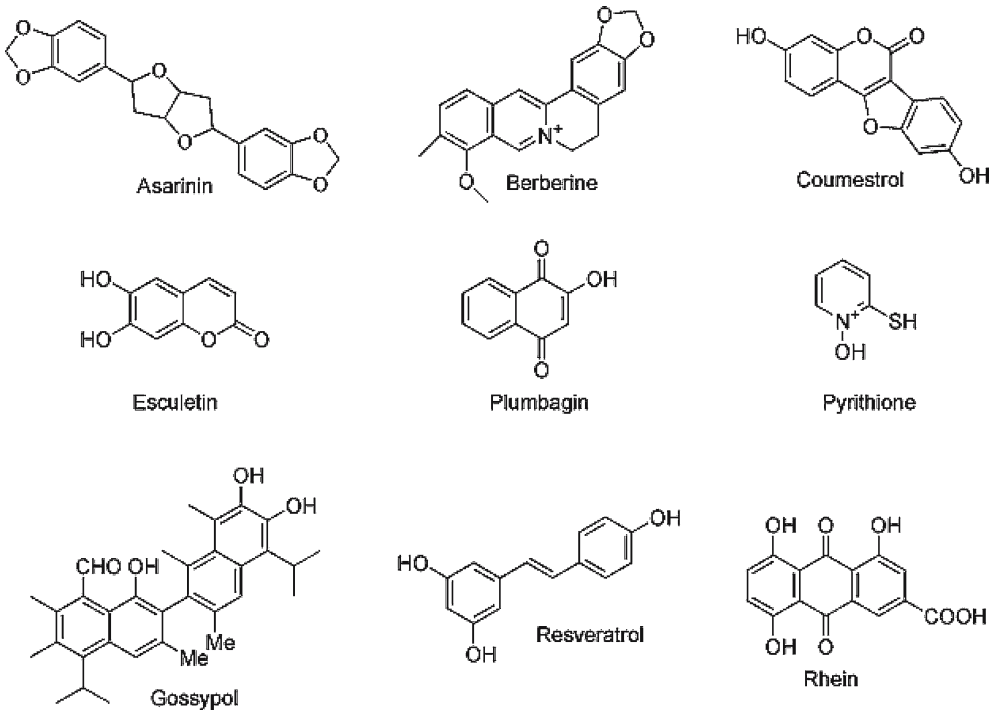
Molecular lipophilicity potential (MLP) is a structure–property descriptor that visualises the lipophilic properties of the molecule on its 3D surface and was calculated by projecting the Broto–Moreau lipophilicity atomic constants on the molecular surface [14]. The virtual log P, Broto log P and lipole, of all single molecules and complexes were evaluated by Vega ZZ software [6, 7]. Vega ZZ software was used to produce figures.

The correlations between different calculated values and activities were examined by the Gretl software package [15].

3 Results and discussion

The antibacterial activity of a series of plant antimicrobials was measured in the absence and in the presence of two different MDR inhibitors [1]. The aim of this study was to find a rationale for interesting observations found in [1]. It was shown previously that modulators of efflux pump activity may have an affinity for antibacterial and anticancer drugs [2]. Here, we have hypothesised that small molecules could interact between themselves and produce complexes to overcome biological barriers such cell efflux pumps and the membranes of Gram-negative bacteria.

The possible complex formation between modulators and antibacterials was examined by molecular modelling. The initial structures of antibacterial and modulator molecules were imported and optimised using Macromodel [9] and MMFFs forcefield [10]. At least five different conformations of each molecule were obtained by a Monte Carlo conformational search and Xcluster analysis [12]. The interaction



Scheme 1

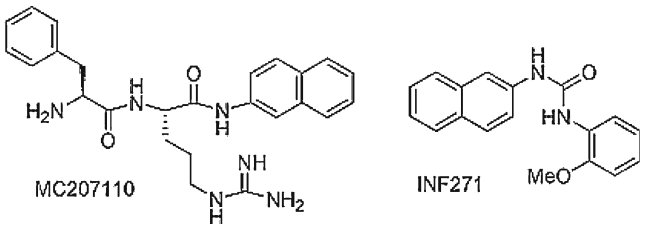
between molecules was investigated using Grid 22 [5] and its docking module Glue 1.0. Both modulators were set as targets with five different conformations for each and binding affinity was tested for several different conformations of antibacterial molecules with rotational freedom for three bonds in ligands.

The complexes with most favourable interaction energies were selected for further analysis: calculation of change of free energy during complexation by Minta [13] and log *P* calculation by Vega ZZ [6, 7]. Generally, a good agreement was observed between interaction energies calculated by Grid and Minta, so potentiation of the drug activity was correlated with interaction energy determined by Grid.

To compare the effects of MDR modulators on activity, the potentiation was calculated by dividing the minimum inhibitory concentration (MIC) of the antibiotic in the presence of a modulator by the value of the MIC where the antibacterial was acting on its own. The increase of activity in the presence of a modulator would give a value larger than 1, while decrease of activity would result in potentiation being lower than 1. If the value of potentiation is 1, a change in activity was not detected.

3.1 Modulation of MDR efflux pump activity in Gram-positive bacterial strains

In the first instance, the correlation between interaction energies and levels of potentiation were examined for two strains of Gram-positive bacteria *S. aureus*, without and with overexpression of the NorA MDR efflux pump. The interac-



Scheme 2

tion energies calculated by Grid and Minta, levels of potentiation, change of log *P* and the levels of potentiation for each of the molecules in the presence of the INF271 modulator are shown in Table 1. Interaction energies between INF271 and all molecules are negative and indicate favourable interactions and therefore suggest that complexes might be formed in an aqueous solution.

3.1.1 Potentiation of antibacterial activity against S. aureus in the absence of the efflux pump NorA

Most antimicrobials had significantly higher activity (higher level of potentiation) in the presence of INF271 against *S. aureus* [1]. This is unexpected, since the *S. aureus* isolate lacks overexpression of the NorA MDR efflux pump and the MDR inhibitor should not have any effect on antibacterial activity. It was proposed that action of other unidentified MDRs [16] could be inhibited and is therefore highly likely to be responsible for this potentiation. Although, the presence of such MDR pumps cannot be overlooked, we are proposing that the formation of an antibacterial-inhibitor complex

could increase antibacterial drug uptake though increasing the lipophilicity.

Drug lipophilicity can be correlated to its permeation in biological systems [17]. Log *P* is the one of the principal parameters to evaluate lipophilicity of chemical compounds and is used as a standard property in determination of potential drug molecules in Lipinski's "rule of 5" [18]. Log *P* coefficients are correlated to passive permeation in biomembranes only when hydrogen-bonding and electrostatic effects are not rate-limiting [19]. MLP calculations can evaluate relationship between conformation and lipophilicity by projecting the Broto–Moreau lipophilicity atomic constants on the molecular surface [14]. Therefore, the MLP calculations were performed on single molecules as well as on all the structures of complexes predicted by Glue.

As shown in Table 1, the change of log *P* is at least 70% as a result of complex formation. Maps of electrostatic potentials for rhein and the rhein–INF271 complex are shown in Fig. 1, suggesting how the lipophilicity is increased through complex formation. The possible complex formation might change the lipophilicity of a complex and its log *P*, consequently it could change the permeability into the cell, therefore overcoming the biological barriers such as MDR pumps or bacterial membranes.

A similar conclusion can be drawn from the potentiation of the antibacterial activity against *S. aureus* in the presence of the MC207110 inhibitor (Table 2). The potentiation is observed for the molecules whose Δ log *P* was above 70%, with the exception of berberine and asarinin, whose log *P* on their own were high (above 4.3).

3.1.2 Potentiation of antibacterial activity against S. aureus overexpressing NorA

Strains of *S. aureus* that overexpress the NorA efflux pump present an additional biological barrier for antibacterial molecules. We have derived an empirical rule that could be used to predict the potentiation of the antibacterial activity in the presence of an MDR inhibitor, the potentiation will be observed if the interaction energy between a modulator and the drug (antibacterial) predicted by Grid and is greater than 9− kcal/mol and smaller than 18 kcal/mol [2]. Although, the rule was an attempt to present a complex picture in a simplified manner, in the set of nine examined molecules in the presence of INF271 the behaviour of six were correctly predicted. The berberine–INF271 pair exhibited a potentiation of 3.32 and the complex had the greatest stabilisation energy of −14.61 kcal/mol (Fig. 2.)

The potentiation for coumestrol, plumbagin and pyrithione was not correctly predicted. The prediction of potentiation of pyrithione has failed due to the small interaction energy; however, pyrithione is a small molecule and consequently the calculated interaction energies would be proportionally smaller. At the present time, we cannot explain the false prediction of potentiation of activity of plumbagin and coumestrol.

Table 1 Potentiation of antibacterial activities of complexes between antibacterials and INF271 modulator against Gram-positive bacteria in correlation with interaction energies and molecular properties

Molecule	Molecular properties						Interaction energies				*S. aureus* [a]		*S. aureus* NorA [a]	
	Log *P*	Δ Log *P*	Lipole	Δ Lipole (%)	Vitual log *P*	Δ Virtual log *P* (%)	E_{grid} kcal/mol	$\Delta\Delta G_c$ (kcal/mol)	$\Delta\Delta G_j$ kcal/mol	ΔH (kj/mol)	MIC (μg/ml) [1]	Potentiation	MIC (μg/ml) [1]	Potentiation
Resveratrol	6.16	156.94	2.17	59.55	5.32	112.58	−10.81	−6.35	−26.56	−22.59	125	2.00	250	4.00
Gossypol	8.94	72.80	1.61	−50.94	6.61	38.79	−18.17	−8.98	−37.61	−41.92	3.12	0.10	1.95	0.25
Coumestrol	7.49	101.07	2.10	21.52	5.25	59.11	−12.35	−3.50	−16.11	−15.60	250	2.00	250	1.00
Rhein	4.25	773.10	1.89	53.74	3.28	552.80	−12.07	−7.17	−30.01	−30.02	4.0	6.45	4	6.45
Plumbagin	4.79	368.76	2.25	11.37	4.02	266.40	−10.14	−6.03	−25.24	−23.17	0.78	2.52	0.31	1.00
Pyrithione	4.23	816.70	1.98	6.74	4.46	361.05	−7.02	−3.93	−16.45	−17.84	0.62	3.88	0.16	2.00
Berberine	8.14	86.16	0.56	−72.32	6.24	45.19	−14.61	−8.61	−36.00	−36.69	250	128.21	3.25	3.32
Esculetin	5.38	232.55	1.50	47.77	3.99	148.58	−7.21	−6.57	−27.50	−26.24	31.25	2.00	15.62	1.00
Asarinin	8.08	87.27	1.55	−22.80	5.52	44.70	−14.85	−8.76	−36.66	−28.71	62.50	4.00	31.25	2.00

The log *P*, lipole and virtual log *P* were calculated by the Broto method implemented in Vega ZZ [6, 7]. Δ values are given as percent change compared to values of antibacterial on its own. E_{grid} is the interaction energy calculated by Grid [5]. ΔΔ G values were estimated by Minta [13] and Δ H was calculated by Macromodel [9] and MMFFs force filed [10] MIC values are reported activities of antibacterials without MDR inhibitor and potentiation was calculated by dividing the MIC of the antibacterial in the presence of MDR inhibitor by the MIC of antibacterial on its own–results taken from [1] [a] it Staphylococcus aureus without and with overexpression of the NorA MDR efflux pump, respectively

Table 2 Potentiation of antibacterial activities of complexes between antibacterials and MC207110 modulator against Gram-positive bacteria in correlation with interaction energies, molecular properties and change of molecular properties compared to single molecule

Molecule	Log *P*	Δ Log *P*	E_{grid} (kcal/mol)	ΔΔ G_J(kcal/mol)	ΔΔ G_c (kJ/mol)	Δ *H*(kJ/mol)	Potentiation	
							S. aureus[a]	*S. aureus* NorA[a]
Resveratrol	3.16	316	−11.58	−24.54	−5.86	−23.84	1.00	1.00
Gossypol	5.93	14.68	−13.68	−47.97	−11.46	−51.45	1.00	1.00
Coumestrol	4.48	20.38	−12.25	−18.25	−4.36	−23.17	1.00	1.00
Rhein	1.25	155.85	−9.32	−38.02	−9.09	−47.35	2.00	6.45
Plumbagin	1.78	74.34	−8.37	−26.42	−6.32	−27.57	2.52	1.00
Pyrithione	1.22	164.64	−7.54	−12.86	−3.08	−20.16	3.88	2.00
Berberine	5.13	17.37	−16.60	−37.27	−8.91	−44.43	8.00	2.01
Esculetin	2.38	46.88	−6.51	−34.49	−8.24	−34.92	1.00	1.00
Asarinin	5.07	17.59	−11.48	−31.78	−7.60	−25.54	2.00	2.00

The Δ log *P* is defined as a percent change of log *P* of a complex compared to values of antibacterial on its own by the Broto method implemented in Vega ZZ [6, 7]. E_{grid} is the interaction energy calculated by Grid [5], ΔΔ *G* values were estimated by Minta [13] and Δ *H* was calculated by Macromodel [9] and MMFFs force filed [10] The potentiation was calculated by dividing the MIC of the antibacterial in the presence of the MDR inhibitor by the MIC of the antibacterial on its own – results taken from [1]
[a] *S. aureus* without and with overexpression of the NorA MDR efflux pump, respectively

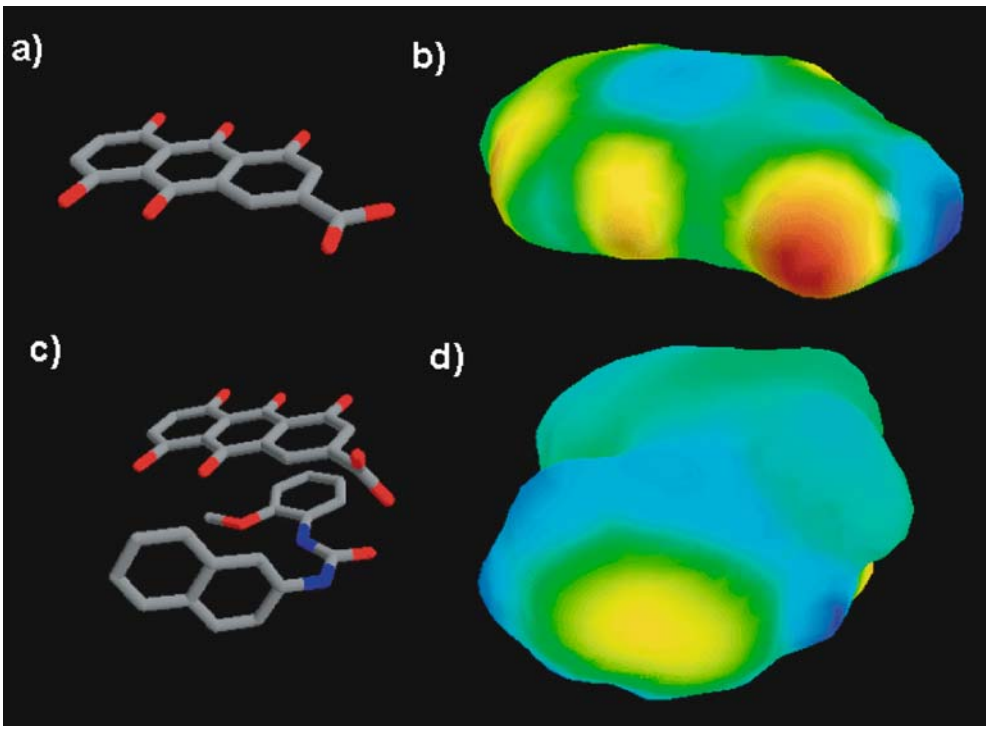


Fig. 1 Structures of rhein (**a**) and rhein–INF271 complex (**b**)and their maps of elestrostatic potentials (**c** and **d**, respectively). Complex was determined using Grid software [5] and structures were optimised using Macromodel [9] and MMFFs force field [10]

A similar combination of interaction energies and log *P* changes could be used to explain the potentiation of antibacterial activity in the presence of the MC207110 modulator (Table 2). For example, berberine and rhein complexes had their interaction energies higher than −9 kcal/mol and their activities were potentiated. However, molecules that had interaction energies higher than −9 kcal/mol and their activities were not potentiated by the presence of MC207110 did not have a significant increase of the log *P* after complex formation to increase uptake. Observed correlations between potentiation and Δ log *P* was 0.6478, while the correlation with interaction energy E_{grid} was only 0.1083.

Figure 2 presents maps of electrostatic potentials for resveratrol and plumabgin, suggesting that the shape of the complex might play a role in MDR modulation. Therefore, we believe that by using a combination of better-defined rules, that include interaction energies, log *P* and shape of a complex, it would be possible to predict the potentiation for an antibacterial drug–MDR modulator pair.

3.1.3 Unusual behaviour of gossypol

The presence of INF271 did not potentiate the activity of gossypol, and it decreased by approximately tenfold in the wild

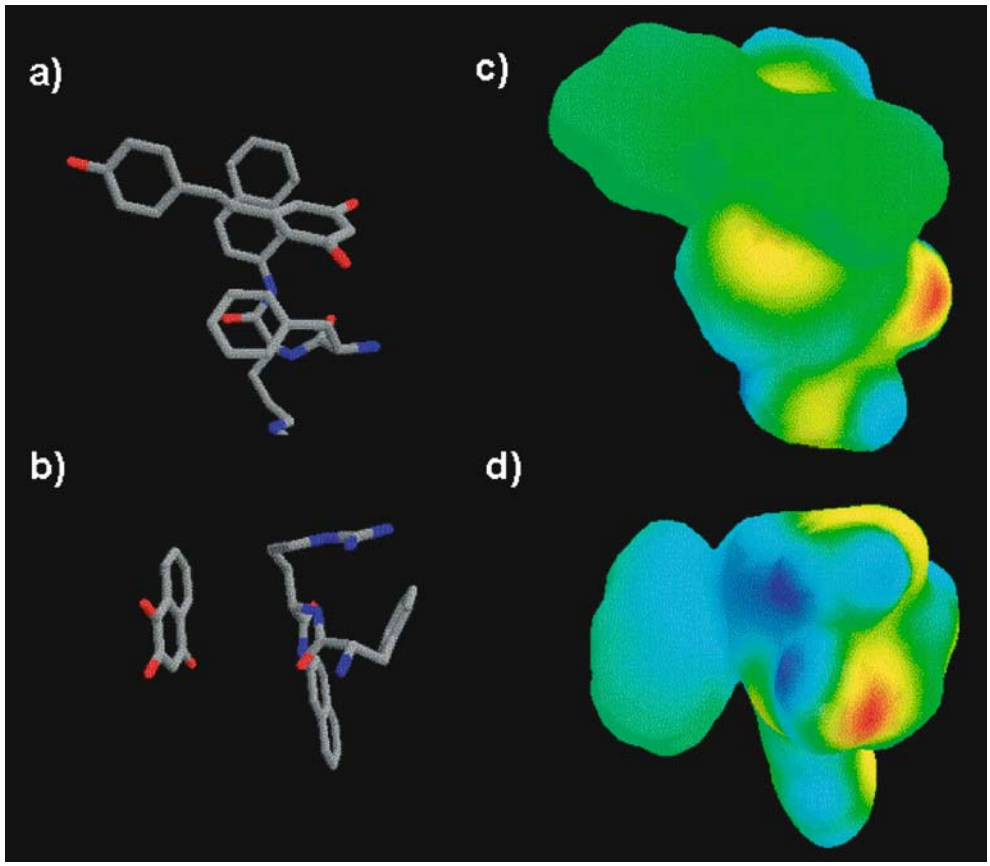


Fig. 2 Structures of resveratrol–MC207110 (**a**) and plumabgin– MC207110 (**b**) complexes andcorresponding maps of elestrostatic potentials (**c** and **d**, respectively). Complexes were determined using Grid software [5] and structures were optimised using Macromodel [9] and MMFFs force field [10]

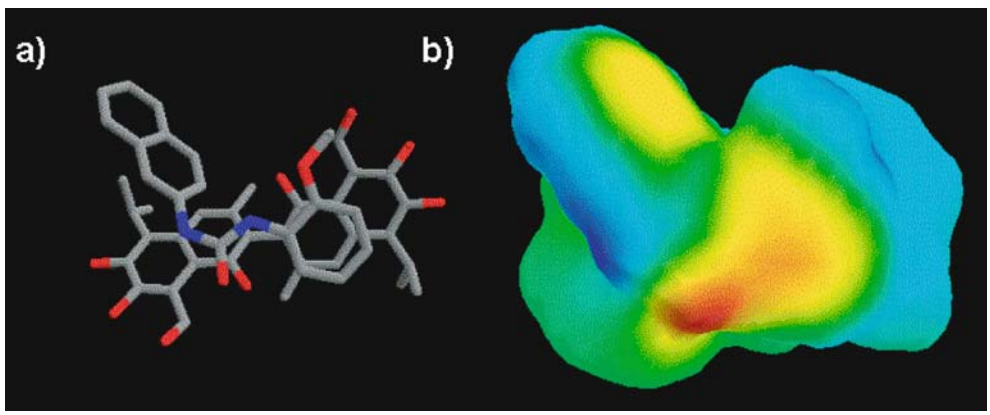


Fig. 3 Structure and map of elestrostatic potentials of gossypol and INF271 complex. Complex was determined using Grid software [5] and structure was optimised using Macromodel [9] and MMFFs force field [10]

type, and fourfold in the NorA strain of *S. aureus* [1]. Similar behaviour was reported with abietic acid and the MDR modulator reserpine [20]. NMR and molecular modelling studies have indicated that a highly interacting complex between reserpine and abietic acid was formed that could decrease activity due to poor availability of abietic acid to act on the drug target. Interestingly, the interaction energy between gossypol and INF271 was −18.17 kcal/mol, indicating that the complex formed between these two molecules (Fig. 3) could be stable and not disassociate at the target site, thus decreasing the activity of the antibiotic in the presence of INF271. In a similar fashion, α-tocopherol has a higher energy of interaction with MDR inhibitors than substrates (−19.06 kcal/mol with GG918) [2] and it has been shown to reverse the effects

Table 3 Potentiation of antibacterial activities of complexes between antibacterials and MC207110 modulator against Gram- negative bacteria in correlation with interaction energies, molecular properties and change of molecular properties compared to the single molecule

MC207110	Δ log *P*	Lipole	E_{grid} (kcal/mol)	Potentiation										
				EC	*EC tolC*	*PA*O1	*PA mexAB*	*SEST*	PS	*XC*	*AT*	*ER*	*ECA*	*SM*
Resveratrol	31.6	4.35	−11.58	4.00	255.10	16.00	16.00	127.88	16.00	32.01	4.00	8.00	128.21	8.00
Gossypol	14.7	2.93	−13.68	4.00	16.01	4.00	2.00	4.00	1.00	16.01	2.00	31.95	2.00	3.99
Coumestrol	20.4	2.72	−12.25	8.00	1.00	2.00	8.00	8.00	2.00	2.00	4.00	1.00	16.01	2.00
Rhein	155.8	1.73	−9.32	80.00	8.00	2.00	4.00	5.00	51.20	80.00	2.00	16.00	16.00	10.24
Plumbagin	74.3	2.98	−8.37	20.00	2.56	16.00	16.00	10.00	5.13	64.10	8.01	10.00	16.03	8.33
Pyrithione	164.6	1.96	−7.54	12.50	25.00	6.40	6.45	3.23	1.56	3.13	12.50	6.25	12.50	12.80
Berberine	17.4	4.05	−16.60	4.00	1.00	2.00	1.00	4.00	4.00	2.00	2.00	2.00	4.00	222.22
Esculetin	46.9	3.61	−6.51	4.00	4.00	2.00	2.00	4.00	4.00	4.00	2.00	4.00	4.00	4.00
Asarinin	17.6	3.81	−11.48	4.00	4.00	2.00	1.00	4.00	4.00	4.00	2.00	4.00	4.00	4.00

The log *P* and lipole were calculated by the Broto method implemented in Vega ZZ [6, 7], Δ values are given as percent change compared to values of antibacterial on its own. E_{grid} is the interaction energy calculated by Grid [5], and Δ *H* was calculated by Macromodel [9] and MMFFs force filed [10] MIC values are reported activities of antibacterials without MDR inhibitor and potentiation was calculated by dividing the MIC of the antibacterial in the presence of MDR inhibitor by the MIC of antibacterial on its own – results taken from [1] *EC Escherichia coli* K-12; *EctolC E. coli tolC mutant PA01 Pseudomonas aeruginosa* PA767; *PAmexAB P.* aeruginosa K119; *SEST Salmonella enterica* serovar Typhimurium; *PS P. syringae* pv. *maculicola* ES4326 *; XC Xanthomonas campestris* XCC528; *; AT Agrobacterium tumefaciens* GV3101 *ER Erwinia rhapontici* Er1 *; ECA Erwinia carotovora* ATCC 358; *SM Sinorhizobium meliloti* Rm1021

Table 4 Potentiation of antibacterial activities of complexes between antibacterials and INF271 modulator against Gram-negative bacteria in correlation with interaction energies, molecular properties and change of molecular properties compared to a single molecule

INF271	Δ log *P*	Lipole	E_{grid}(kcal/mol)	Potentiation										
				EC	*EC tolC*	*PA*O1	*PA mexAB*	*SEST*	PS	*XC*	*AT*	*ER*	*ECA*	*SM*
Resveratrol	156.9	2.17	−10.81	2.00	255.10	4.00	2.00	2.00	2.00	2.00	4.00	4.00	4.00	2.00
Gossypol	72.8	1.61	−18.17	1.00	2.00	2.00	1.00	1.00	1.00	2.00	2.00	4.00	2.00	3.99
Coumestrol	101.1	2.10	−12.35	2.00	0.06	2.00	2.00	2.00	2.00	1.00	2.00	1.00	4.00	4.00
Rhein	773.1	1.89	−12.07	5.00	2.00	2.00	1.00	5.00	25.60	40.00	2.00	2.00	2.00	4.00
Plumbagin	368.8	2.25	−10.14	2.00	1.00	2.00	2.00	2.00	2.56	2.08	2.00	5.12	4.01	2.08
Pyrithione	816.7	1.98	−7.02	2.00	2.00	3.20	2.00	1.00	1.00	1.56	3.13	1.00	2.00	2.00
Berberine	86.2	0.56	−14.61	2.00	2.00	2.00	1.00	2.00	2.00	2.00	2.00	2.00	4.00	2.00
Esculetin	232.5	1.50	−7.21	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Asarinin	87.3	1.55	−14.85	2.00	2.00	2.00	1.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00

The log *P* and lipole were calculated by the Broto method implemented in Vega ZZ [6, 7], Δ values are given as percent change compared to values of antibacterial on its own. E_{grid} is the interaction energy calculated by Grid [5], and Δ *H* was calculated by Macromodel [9] and MMFFs force filed [10] MIC values are reported activities of antibacterials without MDR inhibitor and potentiation was calculated by dividing the MIC of the antibacterial in the presence of MDR inhibitor by the MIC of antibacterial on its own – results taken from [1]
EC E. coli K-12; EctolC E. coli tolC mutant; PA01 - P. aeruginosa PA767; PAmexAB Pseudomonas aeruginosa K119; SEST Salmonella enterica serovar Typhimurium; PS P. syringae pv. maculicola ES4326 XC X. campestris XCC528; AT A. tumefaciens GV3101 ER Erwinia rhapontici Er1; ECA Erwinia carotovora ATCC 358; SM Sinorhizobium meliloti Rm1021

of MDR inhibitors [20]. This supports the hypothesis of the importance of small molecule–small molecule interactions role in overcoming MDR.

3.2 Modulation of antibacterial activity in Gram-negative bacterial strains in the presence of MDR modulators

The potentiation of activity of antibacterials in the presence of MDR inhibitors MC207110 and INF271 are shown in Tables 3 and 4, respectively. In Gram-negative bacteria, the situation is more complex, due to the presence of an additional outer membrane and efflux pumps in some of the bacterial strains. As shown in the previous sections, there is the possibility of complex formation between drugs and inhibitors that could affect the uptake of a drug. However, the same cutoff point of −9 kcal/mol for the interaction energy determined by Grid cannot applied, since the potentiation was observed even if the interaction energy was as low as −6 kcal/mol.

The increase of the lipophilicity and log *P* could be used to explain the potentiation of antibacterial activity in the presence of MDR modulators from different structural classes. A high correlation was observed between the % increase of log *P* and potentiation, for example the correlation coefficient between these two variables was 0.69 in the case of *Escherichia coli* K-12, while the correlation coefficient between potentiation and interaction energy was only 0.27. However, the opposite correlations were observed for *Sinorhizobium meliloti* Rm1021, the correlation coefficient between interaction energy and potentiation was high (0.66), while the correlation coefficient between potentiation and % increase of log *P* was only −0.23.

Similar correlations were observed for the potentiation of drug action in the presence of the INF271 modulator. The potentiation of drug action against *Xanthomonas campestris* XCC528 (*X. campestris)* in the presence of INF271 was in good correlation with the % increase of log *P* (0.59) and a small correlation with interaction energy (−0.13). In the case

of *P. aeruginosa* K1119 (*P. aeruginosa mexAB*) the correlation coefficient between the energy of interaction and potentiation was 0.766 with no correlation with % increase of log *P*.

Although these observations provide general support to the hypothesis, more precise and specific rules for predicting the potentiation of the drug action in the presence of a modulator could be derived from more extensive studies carried out for each strain of Gram-negative bacteria.

4 Conclusion

Complex formation between antibacterial drugs and MDR modulators was predicted by molecular modelling. It was shown that the potentiation of the antibacterial activity could be correlated to interaction energies between complexes and/or log *P* of formed complexes. The unusual decrease in antibacterial activity of gossypol in the presence of INF271 modulator, could be explained by complex formation with strong interactions, thus preventing drug action.

The Grid and Glue software could be used to predict the antibacterial activity potentiation for a drug–inhibitor pair and could find use as a quick screen for new MDR modulators of efflux activity of *S. aureus* overexpressing NorA.

More comprehensive models that include interaction energies and log *P* predictions could be developed to evaluate different MDR modulators and their ability to potentiate drug activity against Gram-negative bacteria.

It is possible that small molecule–small molecule interactions could play an important role in overcoming biological barriers in drug delivery.

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