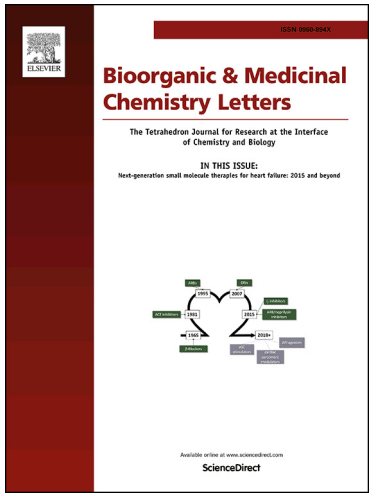
# Accepted Manuscript

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# Design, synthesis and evaluation of a series of 5-methoxy-2,3-naphthalimide derivatives as AcrB inhibitors for the reversal of bacterial resistance

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Running title: 5-methoxy-2,3-naphthalimides as efflux pump inhibitors

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**ABSTRACT**

A series of novel 5-methoxy-2,3-naphthalimide derivatives were designed, synthesized and evaluated for their biological activities. In particular, the ability of the compounds to synergize with antimicrobials, to inhibit Nile Red efflux, and to target AcrB was assayed. The results showed that the most of the tested compounds more sensitized the *Escherichia coli* BW25113 to the antibiotics than the parent compounds **7c** and **15**, which were able to inhibit Nile Red efflux. Significantly, compound **A5** possessed the most potent antibacterial synergizing activity in combination with levofloxacin by 4 times and 16 times at the concentration of 8 and 16 μg/mL, respectively, whilst **A5** could effectively abolish Nile Red efflux at 100 μM. Additionally, target effect of **A5** was confirmed in the outer- or inner membrane permeabilization assays. Therefore, **A5** is an excellent lead compound for further structural optimization.

**Keywords**: 5-Methoxy-2,3-naphthalimide, Antimicrobial resistance, Efflux pump inhibitor, AcrB, Synergism, Inhibition of efflux

The discovery of antibiotics has greatly guaranteed the survival of human beings and improved human life. However, with the widespread use and abuse of antibiotics, multi-drug resistant bacteria have emerged across the world, which has threatened human life and health seriously.[1,2] Up to date a lot of new antibacterial drugs[2-5] and novel therapeutic strategies[1,6-10] have been developed to solve the problem of the bacterial resistance. In particular, over-expression of efflux pump proteins on the membrane of bacteria is one of the major reasons for bacterial multidrug resistance.[6] Among them, the AcrAB-TolC efflux pump widely exists in Gram-negative bacteria, which are characterized by strong conservation and wide array of efflux substrates.[11] Therefore, an effective way to solve the multidrug resistance of Gram-negative bacteria is through the discovery of the efflux pump inhibitors targeting the AcrB transporter.[12]

In the past decades, multiple classes of AcrB efflux pump inhibitors have been reported. For example, the peptidomimetic phenylalanylarginine-β-naphthylamide (PaβN)[13] is the first discovered efflux pump inhibitor with broad-spectrum activity, but its toxicity prevents it from entering clinical trials.[14] Naphthylpiperazines (NMP) can increase the antibacterial activity of several antibiotics and has been one of the most potent efflux pump inhibitors until now. However, its "serotonin agonist'' property seems to be too toxic to be used in clinical practice.[15,16] Another class of the efflux pump inhibitors such as the quinoline derivatives show full activity only at high concentrations but their toxicity is unknown. Another promising class of efflux pump inhibitors are the 5-arylidenehydantoin derivatives.[17] Among them, compound **15**[17] is the best representative, which increases the antibacterial activity of sparfloxacin by 20 fold at 23 μg/mL.

Our group has reported that 4-substituted-2-napthamide derivatives[17,18] inhibit AcrB efflux activity, which are designed and synthesized based on the structure of natural product shikonin.[20] Among them, the representative compound **7c**[19] can increase the antibacterial activity of erythromycin by 8 times at 128 μg/mL. Structural studies upon the efflux pump of *Escherichia coli* have revealed a binding site in AcrB subunit that can accommodate structurally unrelated compounds.[21,22] This is achieved primarily through a hydrophobic pocket that is lined with five phenylalanine residues at the positions 136, 178, 610, 615 and 628, which provide opportunities for multiple hydrophobic and π-π stacking interactions. Molecular docking studies upon the 2-naphthamide inhibitor with the crystal structure of AcrB highlighted a key π-π stacking interaction between the napthamide aromatic ring and the benzyl side chain of Phe626.[18] These structural data, in combination with our previous findings, suggest that a novel 2,3-naphthalimide skeleton is designed by fusing the imide ring of **15** to the strong aromatic bicycles from 4-substituted-2-naphthamide **7c** (Fig. 1). This new scaffold can facilitate hydrophobic alkyl and arylalkyl groups to be appended onto the imide heterocycle, which may exploit the hydrophobic binding sites of AcrB and yield novel and potent inhibitors. In this study, a series of the 5-methoxy-2,3-naphthalimide derivatives were designed and synthesised as efflux pump inhibitors against antibiotic-resistant Gram-negative *E. coli*. Those compounds were tested for their antibacterial activity, synergism with antimicrobials and inhibition of Nile red efflux. Off-target effects such as membrane permeabilization were also investigated.

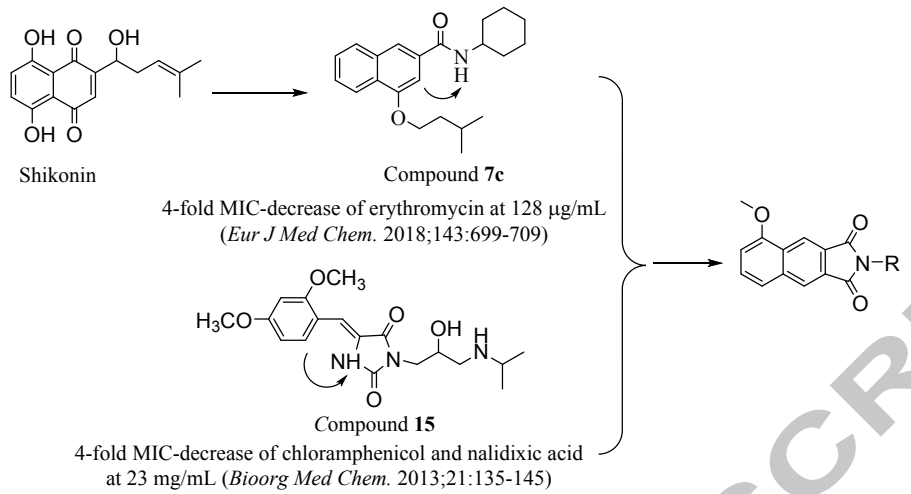
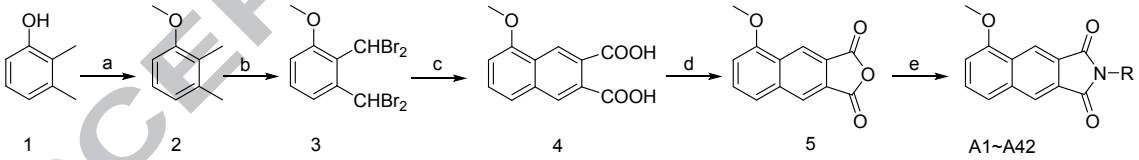



**Fig. 1.** The design of the 5-methoxy-2,3-naphthalimide derivatives.

The 5-methoxy-2,3-naphthalimide derivatives (**A1**~**A42**) were synthesized from the commercially available 2,3-dimethylphenol (**1**) as outlined in **Scheme 1**. Methylation of 2,3-dimethylphenol (**1**) in acetone using methyl iodide ($CH_3I$) at 50 °C gave 1-methoxy-2,3-dimethylbenzene (**2**) in an almost quantitative yield. Bromonation of **2** with N-bromosuccinimide (NBS) in tetrachloromethane ($C_4Cl_4$) in the presence of 2,2′-azobis(2-methylpropionitrile) (AIBN) under reflux conditions produced 1,2-bis(dibromomethyl)-3-methoxybenzene (**3**) in a good yield. Cyclization of **3** with maleic anhydride in the presence of potassium iodide (KI) (4.83g, 30 mmol) in N,N-dimethylformamide (DMF) underwent the Diels-Alder reaction at 70 °C to produce 5-methoxynaphthalene-2,3-dicarboxylic acid (**4**) in a good yield. Finally, dehydration of **4** in acetic anhydride ($Ac_2O$) under reflux generated the 5-methoxynaphtho[*2,3-c*]furan-1,3-dione (**5**), which was followed by reaction with the corresponding amines in pyridine to provide forty-two 5-methoxy-2,3-naphthalimide derivatives (**A1**~**A42**). The newly synthesized compounds were characterized by MS, $^1$H NMR and $^{13}$C NMR. All the spectral data were in agreement with the proposed structures.




A1 R = benzyl
A2 R = 2-chlorobenzyl
A3 R = 4-chlorobenzyl
A4 R = 3-fluorobenzyl
A5 R = 2-methoxybenzyl
A6 R = 4-methoxybenzyl
A7 R = 4-methylbenzyl
A8 R = 4-fluorobenzyl
A9 R = phenyl
A10 R = 2-fluorophenyl
A11 R = 4-fluorophenyl
A12 R = 3-chlorophenyl
A13 R = o-tolyl
A14 R = m-tolyl
A15 R = 2-methoxyphenyl
A16 R = 3-methoxyphenyl
A17 R = 4-methoxyphenyl
A18 R = 4-(tert-butyl)phenyl
A19 R = 2-chlorophenyl
A20 R = 2-isopropylphenyl
A21 R = 2-ethylphenyl
A22 R = 4-isopropylphenyl
A23 R = 4-butylphenyl
A24 R = 2-(trifluoromethyl)phenyl
A25 R = 4-(trifluoromethyl)phenyl
A26 R = 2-(trifluoromethoxy)phenyl
A27 R = 4-(trifluoromethoxy)phenyl
A28 R = 4-ethylphenyl
A29 R = 2-phenethyl
A30 R = 4-fluorophenethyl
A31 R = 3-methoxyphenethyl
A32 R = 2-chlorophenethyl
A33 R = 4-methoxyphenethyl
A34 R = cyclopentyl
A35 R = cyclohexyl
A36 R = hexyl
A37 R = pentyl
A38 R = tert-butyl
A39 R = butyl
A40 R = isopropyl
A41 R = ethyl
A42 R = methyl

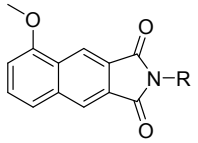
**Scheme 1.** Reagents and conditions: (a) $CH_3I$, acetone, 50°C, 24h (crude product); (b) NBS, AIBN, $CCl_4$, reflux, 24h, 72% for two-step yeild; (c) Maleic anhydride, KI, DMF, 70°C, 8h, 61%; (d) $Ac_2O$, reflux, 2h; (e) Corresponding amine, pyridine, reflux, 12h.

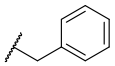
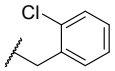
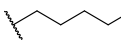
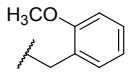
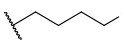
The ability of the target compounds to bind to AcrB and reverse antimicrobial resistance was investigated by assessing reversal of resistance, inhibition of efflux and target specific activity.[23] However, only thirty of the target compounds were characterised because compounds **A3**, **A7**, **A8**, **A14**~**A16**, **A22**~**A24**, **A28**, **A30** and **A32** had limited solubility in dimethyl sulphoxide (DMSO).

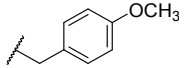
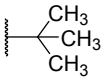
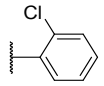
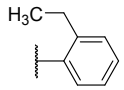
The antibacterial activity of the solubilized compounds was assessed by determining their minimum inhibitory concentrations (MICs) against the wild-type resistant strain of *E. coli* BW25113. As a result, none of the tested compounds showed any antimicrobial activity at the highest test concentration (128 mg/L) against *E. coli* BW2113 nor an isotypic strain lacking the AcrB efflux pump (results not shown).

The target compounds were then tested for their ability to reverse AcrB-mediated resistance. This was performed using an antimicrobial susceptibility assay in the presence of varying concentrations of target compounds together with a panel of antimicrobials using the checkerboard titration assay. The antimicrobials tested here were chloramphenicol, erythromycin, tetraphenylphosphonium and levofloxacin.[24] The data presented in Table 1 shows the activity of the twelve compounds that enhance the sensitivity of *E. coli* BW25113 to erythromycin, chloramphenicol, tetraphenyl phosphonium chloride (TPP) and/or levofloxacin with an antibacterial sensitizing activity equivalent to or better than that of the lead compounds **7c**[19] and **15**.[17] The most potent activity was observed with **A5** and levofloxacin, which lowered the levofloxacin MIC by 16-fold (Table 1). It was noteworthy that benzyl substituted compound **A21** and alkyl substituted compounds **A36** and **A37** displayed broad synergistic activity with all the four antibacterial agents.

**Table** 1. The synergetic effect of 5-methoxy-2,3-naphthalimide derivatives with different antibiotics and TPP against the wild-type drug-resistant strain of *E. coli* BW25113, overexpressing AcrB indicated at (+AcrB). The MIC values of antimicrobials against the same *E. coli* strain with a deletion of the acrB gene are presented for comparison and are indicated as (-AcrB).

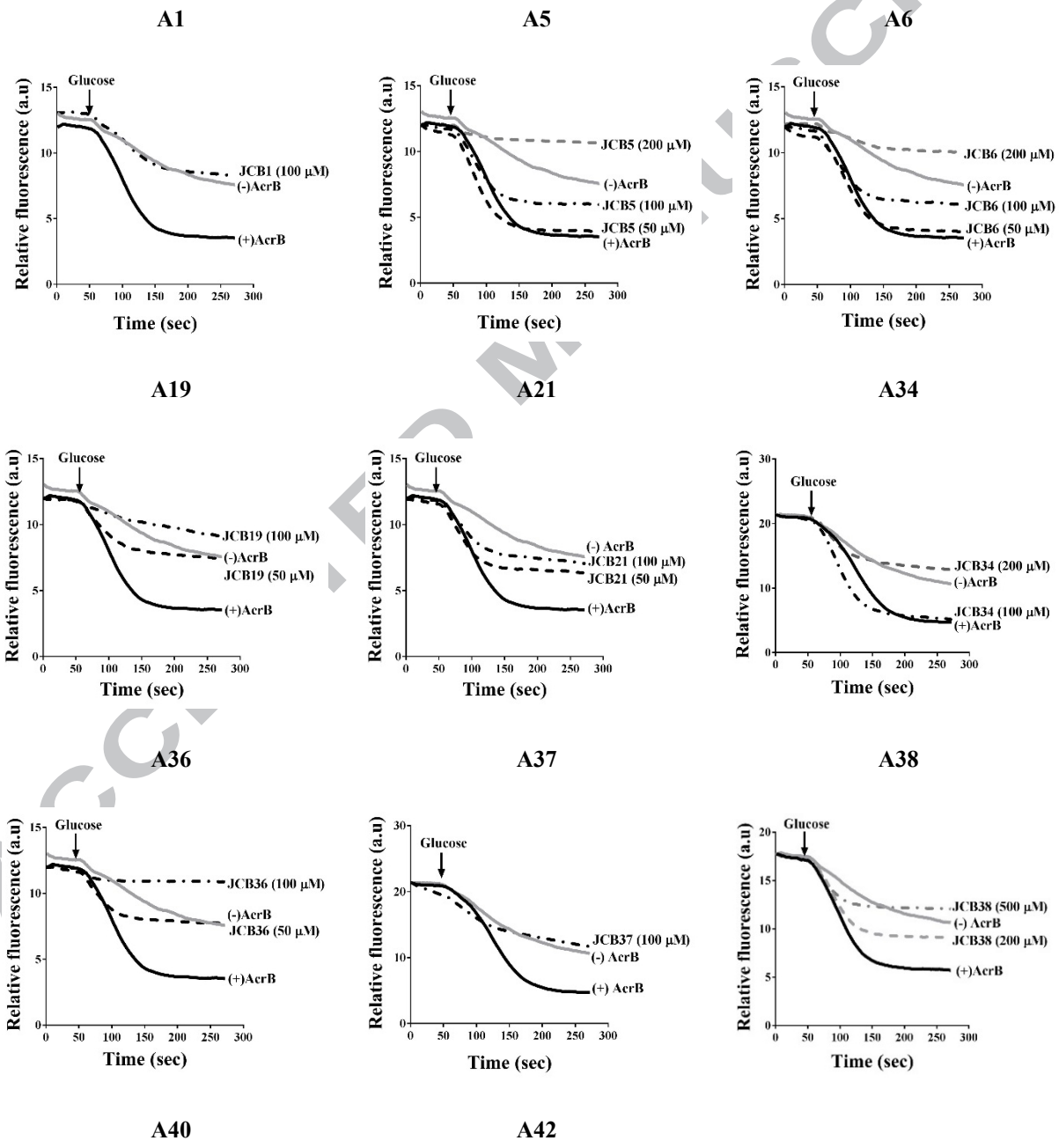



| compound | concentration (μg/mL) | MIC (μg/mL) | | | | compound | concentration (μg/mL) | MIC (μg/mL) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | CAM | ERY | TPP | LEV | | | CAM | ERY | TPP | LEV |
| NONE (+AcrB) | 0 | 16 | 64 | 1024 | 0.063 | NONE (+AcrB) | 0 | 16 | 64 | 1024 | 0.063 |
| NONE (-AcrB) | 0 | 2 | 4 | 32 | 0.0039 | NONE (-AcrB) | 0 | 2 | 4 | 32 | 0.0039 |
| **A1**  | 8 | 16 | 32 | 1024 | 0.063 | | 8 | 16 | 64 | 1024 | 0.063 |
| | 16 | 16 | 32 | 1024 | 0.063 | | 16 | 16 | 64 | 1024 | 0.063 |
| | 32 | 16 | 32 | 1024 | 0.063 | **A34**  | 32 | 16 | 32 | 1024 | 0.063 |
| | 64 | 16 | 32 | 1024 | 0.063 | | 64 | 16 | 16 | 1024 | 0.063 |
| | 128 | 8 | 16 | 512 | 0.063 | | 128 | 16 | 8 | 1024 | 0.063 |
| **A2**   | 8 | 16 | 64 | 1024 | 0.063 | | 8 | 16 | 64 | 1024 | 0.063 |
| | 16 | 16 | 64 | 1024 | 0.063 | | 16 | 16 | 32 | 1024 | 0.063 |
| | 32 | 16 | 64 | 1024 | 0.063 | **A36**  | 32 | 16 | 32 | 512 | 0.063 |
| | 64 | 16 | 64 | 1024 | 0.063 | | 64 | 8 | 16 | 512 | 0.063 |
| | 128 | 16 | 32 | 512 | 0.063 | | 128 | 8 | 8 | 128 | 0.0313 |
| **A5**   | 8 | 16 | 64 | 1024 | 0.016 | | 8 | 16 | 64 | 1024 | 0.063 |
| | 16 | 16 | 64 | 1024 | 0.0039 | | 16 | 16 | 64 | 1024 | 0.063 |
| | 32 | 16 | 64 | 1024 | 0.0039 | **A37**  | 32 | 8 | 32 | 1024 | 0.063 |
| | 64 | 16 | 64 | 1024 | 0.0019 | | 64 | 8 | 32 | 1024 | 0.0313 |
| | 128 | 16 | 64 | 512 | 0.0019 | | 128 | 4 | 8 | 512 | 0.0313 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A6** |  | 8 | 16 | 64 | 1024 | 0.063 | **A38** |  | 8 | 16 | 64 | 1024 | 0.063 |
| | | 16 | 16 | 32 | 1024 | 0.063 | | | 16 | 16 | 64 | 1024 | 0.063 |
| | | 32 | 16 | 32 | 1024 | 0.063 | | | 32 | 16 | 64 | 1024 | 0.063 |
| | | 64 | 16 | 32 | 1024 | 0.063 | | | 64 | 16 | 32 | 1024 | 0.063 |
| | | 128 | 16 | 32 | 1024 | 0.063 | | | 128 | 16 | 32 | 512 | 0.063 |
| **A19** |  | 8 | 16 | 64 | 1024 | 0.063 | **A40** |  | 8 | 8 | 32 | 1024 | 0.063 |
| | | 16 | 16 | 64 | 1024 | 0.063 | | | 16 | 8 | 32 | 1024 | 0.063 |
| | | 32 | 16 | 64 | 1024 | 0.063 | | | 32 | 8 | 32 | 1024 | 0.063 |
| | | 64 | 16 | 64 | 1024 | 0.063 | | | 64 | 8 | 32 | 1024 | 0.0313 |
| | | 128 | 16 | 16 | 512 | 0.063 | | | 128 | 8 | 32 | 512 | 0.0313 |
| **A21** |  | 8 | 16 | 64 | 1024 | 0.063 | **A42** |  | 8 | 16 | 64 | 1024 | 0.063 |
| | | 16 | 16 | 64 | 1024 | 0.063 | | | 16 | 16 | 64 | 1024 | 0.063 |
| | | 32 | 16 | 64 | 1024 | 0.0313 | | | 32 | 16 | 64 | 1024 | 0.0313 |
| | | 64 | 16 | 64 | 1024 | 0.0313 | | | 64 | 16 | 32 | 1024 | 0.0313 |
| | | 128 | 8 | 32 | 512 | 0.0313 | | | 128 | 16 | 32 | 1024 | 0.0313 |

ERY = Erythromycin; CAM = Chloramphenicol; TPP = Tetraphenyl phosphonium chloride; LEV= levofloxacin

The ability of the target compounds identified above to inhibit pump-mediated efflux was determined by measuring the inhibition of the efflux of the fluorescent substrate Nile Red.[25,26] Nile Red is strongly fluorescent in non-polar environments such as the cell membrane, but undergoes a significant decrease in fluorescent quantum yield when in aqueous solutions.[24,25] In the Nile Red efflux assay, **A19**, **A21** and **A36** were found to abolish Nile Red efflux to the level of the pump-deleted (-)AcrB *E. coli* strain at 50 μM, while inhibition of Nile Red efflux was observed at 100 μM for **A1**, **A5**, **A6** and A**37**. However, no efflux inhibition was observed for **A34** at 100 and 200 μM was sufficient to completely abolish Nile Red efflux. **A38**, **A40** and **A42** seemed to have a low affinity for AcrB as only partial inhibition of Nile Red efflux could be seen at 200 μM, while 500 μM of those compounds were needed to completely abolish AcrB-mediated efflux activity (Fig. 2).



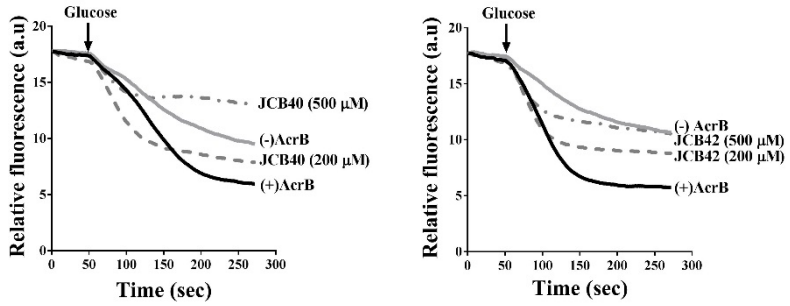
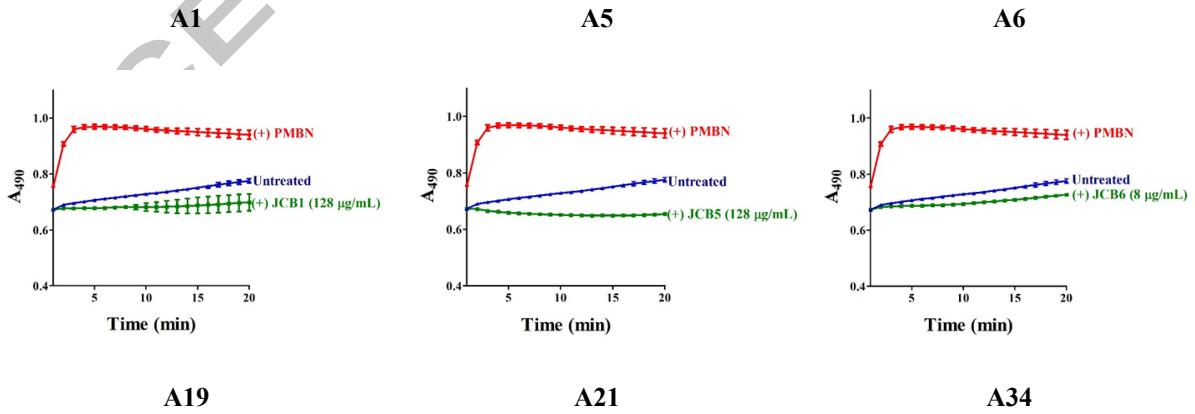



**Fig. 2.** Inhibition of Nile Red efflux**.** Wild-type-resistant strains with active pump (thick black line, (+)AcrB), the strains with a deletion of AcrB (grey line, (-)AcrB) or wild-type strains in the presence of the compounds (dotted lines) were preloaded with Nile Red before the start of fluorescence measurements. Efflux was triggered at 100 sec by the addition of 0.2% glucose (indicated by arrow).[9] Representative fluorescent traces are shown for experiments with different batches of cells done on three different days.

The outer membrane (OM) of Gram-negative organisms acts as a permeability barrier.[27] Hence, the compounds that permeabilize the OM, also synergize with antibiotics as well, and are independent of any inhibition of efflux.[28] The effect on the OM was determined by measuring the rate of nitrocefin hydrolysis when incubated with *E. coli* that expresses β-lactamase.[26] Nitrocefin is a chromogenic β-lactam antibiotic. Hydrolysis of nitrocefin by the bacterial β-lactamase releases a colored compound that can be measured spectroscopically at 490 nm. The rate of nitrocefin hydrolysis is limited by the rate of diffusion across the outer membrane. Hence, an increased rate of hydrolysis of nitrocefin is indicative of outer membrane permeabilization.[29] The rate of nitrocefin hydrolysis in the presence of the tested compounds was comparable to that of the untreated cells (Fig. 3) and much slower than the rate of nitrocefin hydrolysis of cells treated with polymixin B nonapeptide (PMBN), with a well-described ability to permeabilise the Gram-negative OM.[30,31] Thus, none of the compounds compromised the OM in any way, which illustrated that the synergism of the above compounds with the antimicrobials observed was not due to off-target effects such as outer membrane permeabilisation.



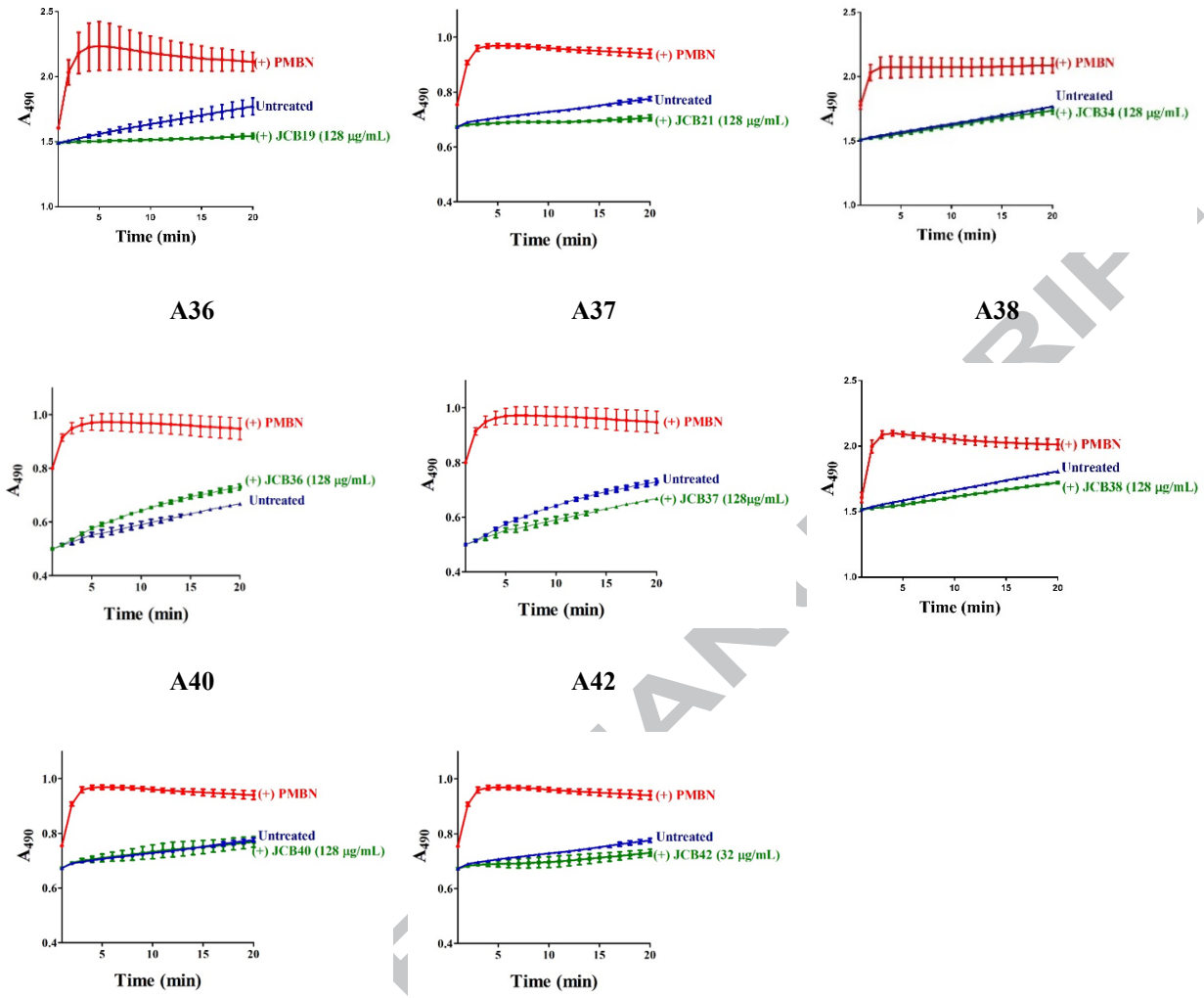
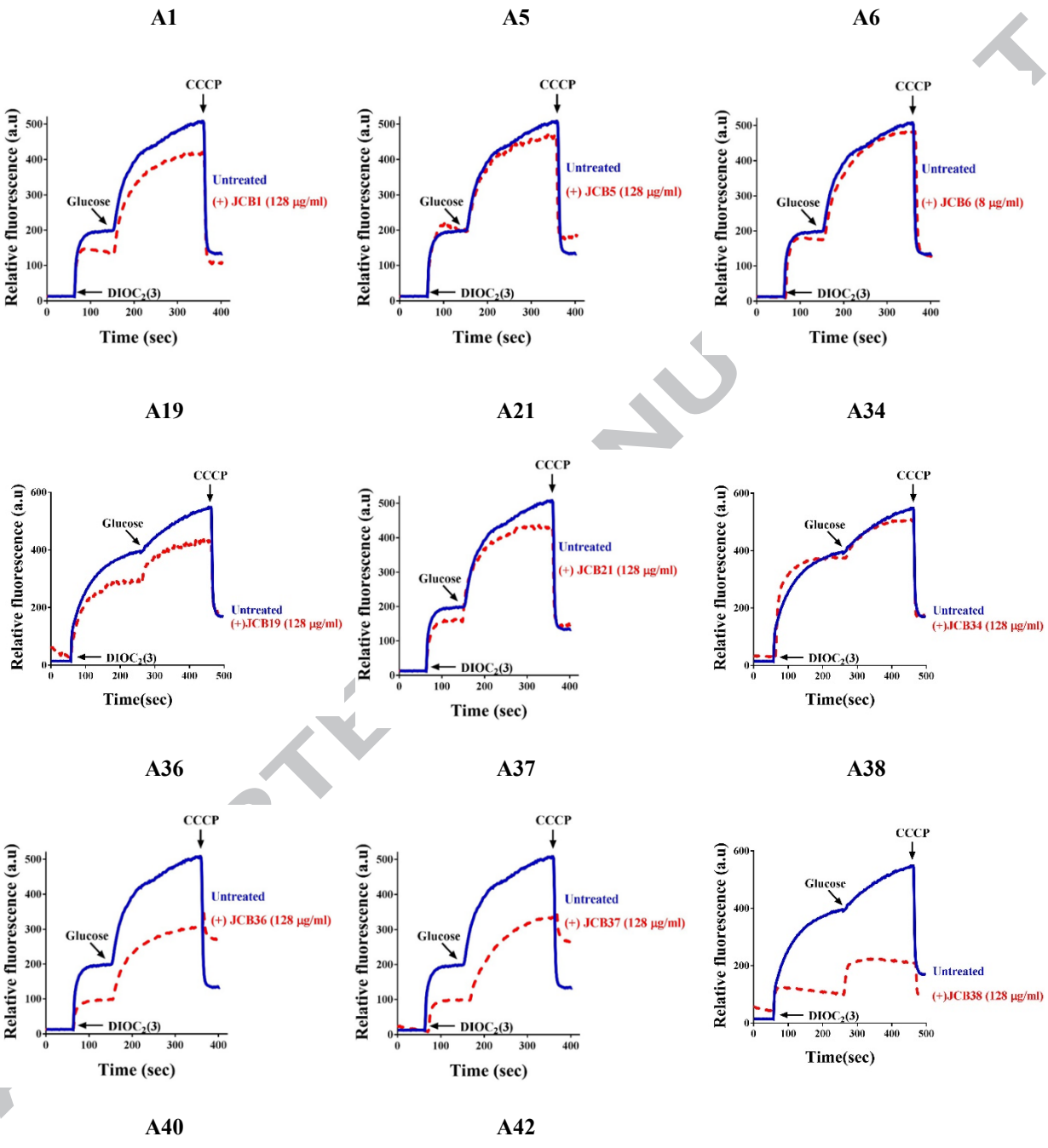



**Fig. 3.** None of the test compounds permeabilize the outer membrane of *E. coli*. Nitrocefin was added to the bacterial cells that received no compound (blue circles) or were treated with the outer membrane permeabilizer polymyxin B nonapeptide (PMBN, red squares) as positive control or the test compounds (green triangles). Nitrocefin hydrolysis by the periplasmic β-lactamase was observed as an increase in $A_{490}$. Compounds that permeabilize the outer membrane, such as PMBN, shows a fast initial rate of nitrocefin hydrolysis in comparison with the untreated the bacterial cells.[9] Representative traces are shown from experiments done in different days.

AcrB is an RND-type transporter that is powered by the proton motive force (pmf) across the bacterial inner membrane.[32] The effect of the active compounds on the integrity of the inner membrane was assessed to distinguish between a direct inhibition of AcrB and the disruption of the proton motive force by permeabilisation of the inner membrane. We previously used the potentiometric probe 3,3-diethyloxacarbocyanine iodide ($DiOC_2(3)$) to measure the magnitude and stability of $\Delta\psi$ in bacterial cells and proteoliposomes[33] and to determine the effect of compounds on the integrity of the bacterial inner-membrane.[18,19] Bacterial cells were energized by the addition of glucose to establish a proton motive force (negative and basic inside the cell), which would lead to an increase in fluorescence associated with aggregation of the $DiOC_2(3)$. Upon addition of the ionophore CCCP, the pmf was dissipated and the fluorescence intensity dropped to the level before addition of glucose. Of all the compounds tested, only **A38** showed a marked decrease in the magnitude of the $\Delta\psi$, while **A36** and **A37** somehow interfered with the ability of CCCP to disrupt the pmf (Fig. 4). These results indicated

that, apart from **A38**, none of the compounds caused disruption of the bacterial inner membrane and hence the synergism with antimicrobials observed was not due to an indirect effect of disrupting the pmf.



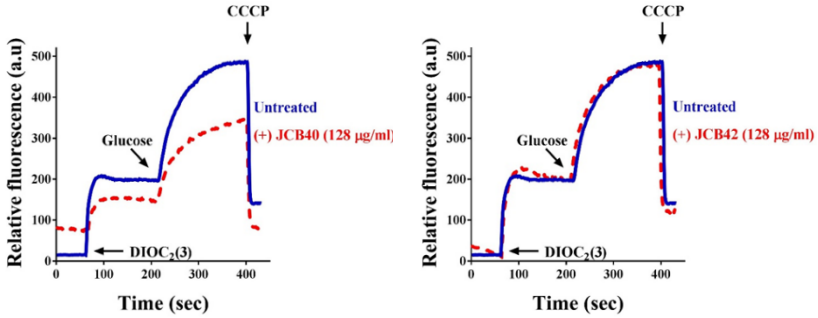



**Fig. 4.** Compounds that effected a reduction in MIC of one or more antimicrobial compounds were subjected to a bacterial inner membrane stability assay to exclude off-target effect such as disruption of the energy source over the inner membrane. Bacterial suspensions were either left untreated (solid blue line) or exposed to appropriate concentration of compounds (broken red line) for 10 min after which the potentiometric probe, DiOC2(3) was added and the fluorescence monitored until it plateaued. Bacterial cells were then re-energized with 0.5% glucose and the establishment of a membrane potential (inside negative) was measured as an increase in fluorescence until it plateaued. The membrane potential was subsequently disrupted by the addition of the proton ionophore CCCP (observed as a sharp drop in fluorescence intensity). After pre-treatment of the bacterial cells with compounds, ΔΨ was maintained for in the presence of all compounds apart from JCB-38, indicating that these compounds do not act on the bacterial inner membrane.

In a 48 h tetrazole (MTT)-based assay, **A5**, **A21** and **A37** were found to show a very low cytotoxicity on human cervical cancer (Hela) cells, with the $IC_{50}$ values of >64 µg/mL. Obviously, their $IC_{50}$ values were much higher than the MIC value of **A5** in synergism of antibacterial agents against the wild-type drug-resistant strain of *E. coli* BW25113.

Docking analysis was performed to explore the binding mode of **A5** to the AcrB (PDB code 5ENO). This molecule was found to bind to the distal binding pocket of AcrB by multiple hydrophobic interactions (Fig. 5). The methoxy group at the 8-position of the naphthalene ring produced hydrophobic interactions with Phe178 and Val 612. The naphthalene ring interacted with Phe136 and Phe615 by π-π stacking, which is critical for the tight binding. Additionally, the *ortho*-methoxybenzyl on the N atom of the imide ring was anchored to the hydrophobic trap to stabilize their interactions (Fig. 6).

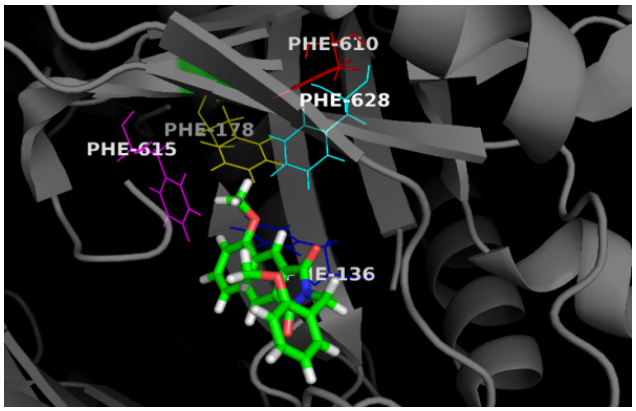



**Fig. 5.** Molecular docking of compound **A5**.

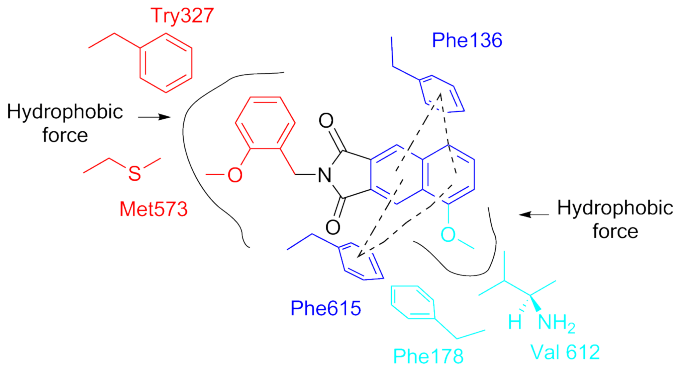



**Fig. 6.** Interactions of compound **A5** with the amino acid residues of AcrB.

Preliminary structure-activity relationship (SAR) study indicated that appending both alkyl (**A36**, **A37**, **A38**, **A40** and **A42**) arylalkyl (**A1**, **A2**, **A5**, **A6**, **A19** and **A21**) side chains into the N atom of the imide ring of the 2,3-naphthalimide skeleton was an effective strategy to inhibit AcrB mediating efflux of antibacterials. The length of the side chains on the N atom of the imide ring appeared to be important for the antibacterial sensitizing activity. For instance, **A30**, **A31** and **A33** with longer linkers were all inactive. Besides, the position and the type of the substituents on the terminal benzene of the side chains were critical to the synergistic activity. For example, the *ortho*-substitution was better than the *para*-substitution (**A5** vs **A6**), and *meta*-substitution (**A19** vs **A12** with no activity) in the synergistic activity. In particular, the methoxy group was the optimal substituent for the synergistic activity. And other electron-donating groups were also promising. Interesting, all fluorine-containing compounds such as **A4**, **A11**, **A24**, **A25**, **A26** and **A27** were inactive in the antibacterial sensitizing activity, probably due to the electronegativity of fluorine atom damaging the π-π stacking interaction. We also observed activity with a variety of appended alkyl groups including cyclohexyl (**A34**), hexyl (**A36**), pentyl (**A37**), tertiary butyl (**A38**), isopropyl (**A40**) and methyl (**A42**) groups. Together those findings highlight that AcrB can bind a variety of structurally diverse structures, and the 5-methoxy-2,3-naphthalimide pharmacophore represents a valuable scaffold for the chemical development of new efflux inhibitors to target multi-drug resistance in Gram negative bacteria.

In conclusion, a series of novel 5-methoxy-2,3-naphthalimide derivatives were designed, synthesized and evaluated for their inherent antibacterial activity, synergism with antimicrobials and inhibition of efflux-pump driven substrate transport while off-target effects such as inner- or outer membrane permeabilization were also ruled out. This study identified five 5-methoxy-2,3-naphthalimide derivatives that displayed synergism to at least one antibacterial with potency equivalent to or better than the 4-fold synergism reported for parent compounds **7C** (erythromycin at 128 μg/mL) and **15** (chloramphenicol and nalidixic acid at 23 μg/mL), namely **A1**, **A5**, **A34**, **A36** and **A37**. Eleven compounds were identified to be able to inhibit substrate efflux, among which, **A19**, **A21** and **A36** exerted strong activity at 50 μM, and **A1**, **A5**, **A6** and **A37** also showed activity at 100 μM. However, complete inhibition of substrate transport by **A40** and **A42** could only be achieved at relatively high concentrations (500 μM) indicating a lower affinity for the AcrB transporter compared to the other test compounds. Hence, the modest effects on the MIC values of antimicrobials observed in the presence of the two compounds were most probably due to competition. **A36** was able to lower the MIC of all substrates tested at 128 μg/mL, with the most potent activity against for erythromycin at 16 μg/mL and were able to completely abolish Nile Red efflux even at 50 μM. **A5** possessed the most potent antibacterial sensitizing activity for levofloxacin by 4 and 16 times at 8 and 16 μg/mL, respectively. Only a handful of AcrB inhibitors reported so

far had the same synergistic antibacterial effect at concentrations as low as 8 and 16 μg/mL. Therefore, **A5**, **A21**, **A36** and **A37** are excellent lead compounds for further structural optimization.

**Conflict of interest**

The authors declare that this study was carried out only with public funding. There is no funding or no agreement with commercial for profit firms.

**Acknowledgements**

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**A. Supplementary material**

Supplementary data associated with this article can be found, in the online version, at http://

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**Graphical Abstract:**

**Design, synthesis and evaluation of a series of 5-methoxy-2,3-naphthalimide derivatives as AcrB inhibitors for the reversal of bacterial resistance**

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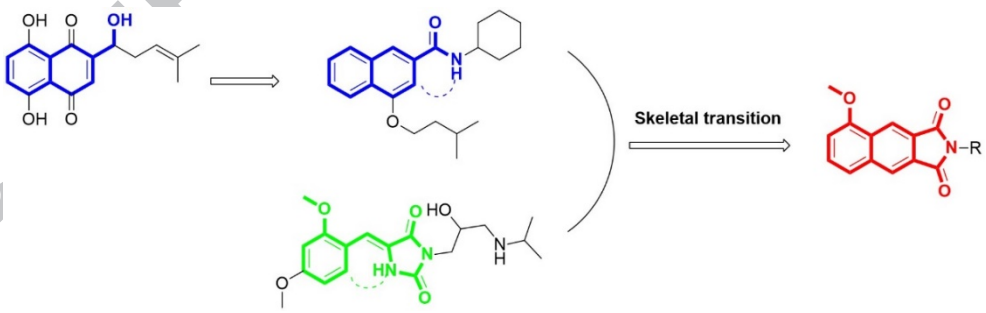


**Research Highlights**

> 5-Methoxy-2,3-naphthalimide derivatives were designed and synthesized. > They were evaluated for their AcrB inhibitory activity and target specific activity. > Twelve derivatives could enhance the sensitivity of antibiotics to strains. > **A5** showed the most potent antibacterial sensitizing activity.