Efflux inhibition by selective serotonin reuptake inhibitors in *Escherichia coli*

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**Objectives:** To evaluate the antimicrobial and synergistic (hypothetically due to the inhibition of efflux pumps) effects of selective serotonin reuptake inhibitors (SSRIs) in *Escherichia coli* strains overproducing various resistance–nodulation–division (RND) efflux pumps.

**Methods:** MICs of various SSRIs and of clinically relevant antibiotics in the presence and absence of sertraline were determined for *E. coli* strains overproducing the RND efflux pumps AcrAB, AcrEF, MdtEF and MexAB. The effect of sertraline on Nile red efflux was evaluated in a real-time efflux assay. Expression of *marA* and *acrB* was monitored using quantitative RT–PCR.

**Results:** In MIC assays there was limited synergy of sertraline with tetracycline, oxacillin, linezolid and clarithromycin, depending on the individual pump overexpressed and on whether rich or minimal medium was used. Sertraline, as the most potent SSRI with regard to bacterial growth inhibition, led to rapid dose-dependent Nile red efflux inhibition, and was also found to increase the expression of *marA* and *acrB*.

**Conclusions:** A possible explanation for the discrepancy between the MIC and real-time efflux assays was that sertraline is a weak inducer of *marA* and *acrB*, thereby reducing its initial antibacterial and sensitizing effects over time. The results indicate that sertraline may be useful as a model efflux pump inhibitor for in vitro short-term experiments in *E. coli*, but is unlikely to be clinically useful as a co-drug against Gram-negative bacteria.

**Keywords:** multidrug efflux, AcrAB-ToLC, SSRIs, sertraline, NR, substrate binding pocket

**Introduction**

Antibacterial resistance is increasing dramatically and the pipeline for new drugs appears almost empty.¹ A solution to this problem could be the development of broad-range potentiators/sensitizers of existing antibiotics, including inhibitors of multidrug efflux pumps.² An additional strategy is the exploitation of synergistic properties of so-called ‘non-antibiotics’, drugs that are already used in human medicine for purposes other than antibacterial treatment but have intrinsic antimicrobial properties and/or are capable of potentiating the activity of existing antibiotics (coined ‘helper compounds’).³

Interestingly, several of the so-called ‘non-antibiotics’ belong to the group of psychotropic drugs. Phenothiazines, for example, have intrinsic antimicrobial activity in a wide range of bacteria and some were shown to be synergistic with selected antibiotics, possibly through drug efflux inhibition.⁴

Selective serotonin reuptake inhibitors (SSRIs) have been studied as ‘non-antibiotics’ in Gram-positive and Gram-negative bacteria.⁵,⁶ Among the SSRIs, sertraline was found to potentiate the activity of fluoroquinolones, presumably via inhibition of efflux pumps.⁶

Here, we reassess the antibacterial and sensitizing activity of SSRIs using *E. coli* strains with overexpression of defined multidrug efflux pumps. We show that sertraline has the properties of a resistance–nodulation–division (RND) efflux pump inhibitor (EPI) in *Escherichia coli*, but has only limited activity as a potentiator/sensitizer of existing antibacterial drugs in MIC assays, possibly due to induction of efflux pumps.

**Materials and methods**

**Strains and culture techniques**

All strains are derivatives of *E. coli* K-12 AG100 (Table 1). The strains were grown at 37°C at 200 rpm in 20 mL of LB medium (containing 1%...
were repeated at least three times. The gene expression studies is available at JAC Online. All necessary steps were performed according to a detailed protocol that has been described elsewhere. To quantify the potency of sertraline in strain 2-DC14PS, while the MICs of the other SSRIs were partly restored (Table 1), suggesting that the tested SSRIs are transported by the E. coli AcrEF-ToIC pump.

No increased SSRI MICs were observed in E. coli DKO20/1 as well as in the second MdtEF-TolC overproducing strain DKO1/17 (containing an MdtF V610F mutation responsible for increased linezolid resistance), suggesting that the tested SSRIs are not extruded by the MdtEF-ToIC pump. Expression of the Pseudomonas aeruginosa pump genes mexAB-oprM from a plasmid in E. coli E12 yielded sertraline MIC values that were slightly higher than those seen in the AcrAB-inactivated strain 1-DC14PS, while the other SSRI MICs were unaffected (Table 1).

Sertraline inhibits NR efflux

We initially performed whole cell accumulation assays using ethidium bromide, Phe-Arg-β-naphthylamide (PAβN) and NR as substrates. Sertraline (at 0.5× MIC) gave enhanced dye accumulation measured over 30 min in E. coli 3-AG100 (ethidium bromide, 1.2-fold; PAβN, 1.2-fold; and NR, 3.8-fold), but not in AcrAB-inactivated E. coli strain 1-DC14PS. Similar results were obtained with paroxetine, but not with citalopram and fluoxetine (data not shown). At higher concentrations (MIC or above), accumulation assays with all SSRIs showed enhanced dye accumulation in both efflux-pump-overexpressing and efflux-pump-inactivated strains (data not shown).

In order to demonstrate that the effects at subinhibitory concentrations were specifically due to efflux inhibition, we opted to examine the effect of sertraline on NR efflux. As shown in Figure 1, sertraline inhibited NR efflux in E. coli 3-AG100 in a dose-dependent manner. Based on estimated IC₉₀ values (Table 1), efflux inhibition occurred below the sertraline MIC in 3-AG100 and 2-DC14PS (100 μM) and above the sertraline MIC in DKO20/1, DKO1/17 and E12/pMexAB-OprM (Table 1). None of the other tested SSRIs showed significant NR efflux inhibition in the different RND-efflux-pump-overexpressing strains at a concentration of 100 μM (data not shown).

Limited synergy in MIC assays

None of the antibiotic MICs—except for oxacillin in 3-AG100—could be reduced by ≥4-fold in the efflux-pump-overexpressing strains using LB medium (Table 2). This was in contrast to the excellent inhibition of NR efflux by sertraline in 3-AG100 and

### Table 1. Bacterial strains used in the study, intrinsic activity of SSRIs as measured by MIC assays in rich (LB) or minimal (MMM) medium, and the concentration of sertraline (in μM) required to inhibit NR efflux by 90% (IC₉₀)

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant genotype</th>
<th>Reference</th>
<th>MIC (μM)</th>
<th>Sertraline IC₉₀ (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>citalopram</td>
<td>fluoxetine</td>
</tr>
<tr>
<td>3-AG100</td>
<td>acrAB+</td>
<td>13</td>
<td>&gt;800</td>
<td>&gt;800</td>
</tr>
<tr>
<td>1-DC14PS</td>
<td>ΔacrAB</td>
<td>14</td>
<td>&gt;800</td>
<td>200</td>
</tr>
<tr>
<td>2-DC14PS</td>
<td>ΔacrAB ΔacrEF+</td>
<td>14</td>
<td>ND</td>
<td>400</td>
</tr>
<tr>
<td>DKO20/1</td>
<td>ΔacrAB ΔacrF mdtEF+</td>
<td>9</td>
<td>ND</td>
<td>100</td>
</tr>
<tr>
<td>DKO1/17</td>
<td>ΔacrAB ΔacrF mdtEF V610F+</td>
<td>9</td>
<td>ND</td>
<td>50</td>
</tr>
<tr>
<td>E12/pMexAB-OprM</td>
<td>ΔacrAB ΔacrF mexAB-oprM+</td>
<td>15</td>
<td>ND</td>
<td>200</td>
</tr>
<tr>
<td>1-DC14PS</td>
<td>ΔacrAB ΔacrF mdtEF+</td>
<td>9</td>
<td>ND</td>
<td>200</td>
</tr>
</tbody>
</table>

ND, not done.

Tryptone, 0.5% yeast extract and 1% NaCl) or in modified MOPS minimal medium (MMM; the original Neidhardt medium amended with glucose (0.5% in the MIC assays and 0.15% in the preparations for RT-PCR) and 100 μg/mL L-arginine).

**Chemicals**

All SSRIs were obtained from Chemos GmbH (Regenstauf, Germany). All other chemicals were obtained from Sigma–Aldrich (Taufkirchen, Germany).

**Susceptibility testing**

The MICs of several substrates alone and in combination with various SSRIs were determined in a 96-well microtitre plate following standard procedures.

**Real-time Nile red (NR) efflux assay**

All necessary steps were performed according to a detailed protocol that has been described elsewhere. To quantify the potency of sertraline in the different strains, the IC₉₀ (concentration that leads to ~90% efflux inhibition) medians of three experiments were determined based on a 2-fold dilution series from 50 through 400 μM.

**Gene expression studies**

Relative expression of E. coli genes gapA, acrB and marA with and without sertraline after growth in MMM was quantified by real-time PCR following reverse transcription using standard procedures (a detailed protocol for the gene expression studies is available at JAC Online). All experiments were repeated at least three times.

### Results and discussion

**Sertraline is the most potent antimicrobial agent of all tested SSRIs**

Among the four SSRIs tested, sertraline was the most potent antimicrobial compound (Table 1), regardless of the medium used (MIC 200 μM). Strain 1-DC14PS was more sensitive than strain 3-AG100, indicating that the tested SSRIs are substrates of AcrAB-ToIC. Sertraline MICs were completely restored in strain 2-DC14PS, while the MICs of the other SSRIs were partly restored (Table 1), suggesting that the tested SSRIs are transported by the E. coli AcrEF-ToIC pump.

Limited synergy in MIC assays

None of the antibiotic MICs—except for oxacillin in 3-AG100—could be reduced by ≥4-fold in the efflux-pump-overexpressing strains using LB medium (Table 2). This was in contrast to the excellent inhibition of NR efflux by sertraline in 3-AG100 and
2-DC14PS. Sertraline potentiated the antibacterial action of tetracycline, rifampicin and clarithromycin in the efflux-deficient strain 1-DC14PS more than in the acrAB-overexpressing strain 3-AG100, suggesting a mode of action independent of efflux pump inhibition.

Of note was the discrepancy of a 4-fold linezolid MIC reduction but poor NR efflux inhibition in the DKO1/17 strain.

To resolve the discrepancy between the results of the efflux assays and the MIC assays, we focused our attention on the medium, since the considerable difference between the use of potassium phosphate buffer in the efflux assays and the use of the rich medium LB in the MIC assays was quite obvious.

Using MMM instead of LB for the selected strains 3-AG100 and 1-DC14PS, sertraline potentiated the activity of tetracycline, linezolid and clarithromycin by ≥4-fold in strain 3-AG100 (Table 2).

Previous observations have shown that the choice of medium affects the expression of efflux-relevant genes in *E. coli*. This may have important implications for the prediction of antibiotic efficacy in the clinical setting, since MICs are generally determined in a nutrient-rich and complex medium, such as Mueller–Hinton or LB, whereas the nutrient content in the human body varies substantially from the conditions in a 96-well plate. We suggest that growth media other than LB may be considered in *E. coli* antibiotic synergy studies to improve their sensitivity in detecting potentially relevant effects.

While inhibition of the AcrAB-ToIC efflux pump by sertraline (at 0.5 × MIC) in MMM was highly likely in strain 3-AG100 with regard to tetracycline and linezolid, as demonstrated by very moderate MIC changes in 1-DC14PS, clarithromycin displayed 4-fold reduced MICs in both strains, suggesting that other mechanisms are at least partly responsible for the synergistic effect of sertraline with this compound. Such an additional mode of action—namely membrane permeabilization—has already been described for the EPI PAβN.

Although growth in MMM enhanced the antibiotic-potentiating effect of sertraline in strain 3-AG100, some substrates of AcrAB-ToIC—namely levofloxacin, chloramphenicol and rifampicin—benefited rather poorly from this EPI. A similar phenomenon has been reported for PAβN, which is a broad-

![Figure 1](https://academic.oup.com/jac/article-abstract/66/9/2057/769164)
spectrum EPI that potentiates the activity of a wide variety of antimicrobials, but not of ethidium. We hypothesize this could be due to multiple preferential substrate binding sites within the AcrB binding pocket and that sertraline might only block one that interacts with a specific subgroup of compounds.

Expression of acrB and marA is increased in the presence of sertraline

We also wanted to address the issue that the different time windows (minutes in the efflux assays and hours in the MIC assays) could be an important factor—hypothetically due to alteration of gene expression—in the discrepancy that was observed between the effect of sertraline in the MIC tests and in the efflux assays, since even the use of MMM caused only a moderate and rather selective increase in antibacterial synergy. We thus compared the expression of acrB and marA genes upon incubation in MMM with and without sertraline using quantitative RT-PCR. When a cut-off of \( \geq 2 \)-fold gene overexpression of the sertraline-containing samples versus the sertraline-free control was applied, meaningful changes could only be detected in the samples containing 100 \( \mu \)M sertraline; 2.6-fold acrB overexpression was detected after a sertraline exposure of 120 min and 2.9-fold marA overexpression was detected after a sertraline exposure of 30 min.

While the changes were not dramatic, the results nevertheless indicate that EPIs can induce the expression of efflux pumps and thus counteract their efficacy—a fact that should be considered when novel EPIs are developed.

Concluding remarks

While sertraline was found to be an interesting model compound for RND efflux pump inhibition, the peak plasma levels that were reported from the literature are well below the concentrations that are needed to block substrate efflux or exert any synergistic effects. Therefore, the use of it as a ‘helper compound’ that potentiates the activity of other antibiotics in the clinic is currently not feasible. However, it is not known whether sertraline concentrations in defined tissues or cells may be higher than those measured in plasma and, therefore, the drug at standard doses could still be useful for enhancing local efficacy of a suitable antibiotic. Also, it cannot be excluded that higher doses of a metabolite of sertraline could be administered, since it might have a better antimicrobial and/or synergistic activity but with less untoward effects on the CNS.

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Supplementary data

A detailed protocol for the gene expression studies is available at JAC Online (http://jac.oxfordjournals.org/).

References