Selektif serotonin geri alım inhibitörlerinin tek başına ve flukonazol ile kombine halde antifungal ve antibiyofilm aktiviteleri

Antifungal and antibiofilm activities of selective serotonin reuptake inhibitors alone and in combination with fluconazole

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ABSTRACT

INTRODUCTION: *Candida* spp. are clinically important pathogens that cause the difficulties for treatment by biofilm formation. Considering antifungal resistance rates and the limitations in discovery of new antifungals, it's getting more important the antifungal and antibiofilm effects of various drugs which are using for different therapeutic purposes. The main goal of our study was to determine antifungal and antibiofilm effects of selective serotonin reuptake inhibitors (SSRIs) sertraline (SRT), paroxetine (PRX), fluoxetine (FLX), alone and in combination with fluconazole (FLC), against *Candida* spp.

METHODS: Twenty *Candida* spp. strains isolated from clinical samples at Ege University Hospital, were identified by Dalmat method and matriks assisted laser desorption ionization time of flight mass spectrometry. Minimum inhibitory concentrations of SSRIs and fluconazole were detected by broth microdilution method. Synergistic interactions between SSRIs and fluconazole were investigated by checkerboard assay. The antibiofilm effects of SSRIs were determined by spectrophotometric microplate method.

RESULTS: Five different *Candida* spp. (*Candida albicans*, *Candida glabrata*, *Candida krusei*, *Candida tropicalis*, *Candida parapsilosis*) were identified among the isolates. The MIC ranges of SSRIs were found as 16-512 µg/mL. It was detected that sertraline was the agent have highest antifungal effect. The antibiofilm efficacy of fluoxetine was found to be higher than other SSRIs. Additionally, fluoxetine and paroxetine showed synergistic effect with fluconazole in thirteen and ten isolates, respectively. Nine isolates were detected as moderate biofilm producer, four isolates were identified as strong biofilm producer. *C. parapsilosis* strains showed higher biofilm production than other species. Fluoxetine and sertraline alone, at MIC/2, inhibited mature biofilms in six and five isolates, respectively while paroxetine was found to increase biofilm formation in seven isolates.

DISCUSSION AND CONCLUSION: This study revealed that sub-MICs of SSRIs could have antifungal and antibiofilm effects. The antidepressants sertraline and fluoxetine, alone and in combination with antifungals are considered to may have therapeutic potential for combating fungal infections.

Keywords: *Candida* spp., Fluconazole, EUCAST, Synergistic effect, antibiofilm

ÖZ

GİRİŞ ve AMAÇ: Klinik açıdan önemli fungal patojenlerden olan *Candida* türleri, biyofilm üretme kapasiteleriyle tedavide zorluklara yol açmaktadır. Antifungal direnç oranları ve yeni antifungallerin keşfinin sınırlılığı göz önüne alındığında, farklı terapötik amaç için kullanılan çeşitli ilaç moleküllerinin antifungal ve antibiyofilm etkileri daha fazla önem kazanmaktadır. Çalışmamızda, seçici serotonin geri alım inhibitörlerinin (SSRI) sertralin (SRT), paroksetin (PRX), fluoksetinin (FLX), tek başına ve flukonazolle kombine halde *Candida* türlerine karşı antifungal ve antibiyofilm etkilerinin belirlenmesi amaçlanmıştır.

YÖNTEM ve GEREÇLER: Ege Üniversitesi Hastanesi'nde klinik örneklerden izole edilen 20 *Candida* spp. kökeni Dalmau metodu ve Matriks aracılı lazer dezorpsiyon/iyonizasyon uçuş zamanı kütle spektrometresi kullanılarak tanımlandı. SSRI moleküllerin ve flukonazolün minimum inhibitör konsantrasyon değerleri sıvı mikrodilüsyon yöntemiyle belirlendi. Flukonazol ve SSRI moleküllerin sinerjistik etkileşimleri dama tahtası metoduyla araştırıldı. SSRI ajanların antibiyofilm etkinlikleri spektrofotometrik mikropilaka yöntemiyle değerlendirildi.

BULGULAR: Yirmi izolat arasında *Candida albicans*, *Candida glabrata*, *Candida krusei*, *Candida tropicalis*, *C. parapsilosis* olmak üzere beş farklı tür belirlendi. SSRIs moleküllerinin MİK değerlerinin 16-512 µg/mL aralığında değiştiği saptandı. SSRI ajanlardan sertralinin yüksek antifungal etkisi gözlenirken, fluoksetinin antibiyofilm etkinliğinin diğer ajanlardan daha yüksek olduğu belirlendi. Fluoksetin ve paroksetin moleküllerinin flukonazolle kombinasyonlarının sırasıyla on üç ve on izolat üzerinde sinerjistik etkisi belirlendi. Dört izolatın güçlü, dokuz izolatın ise orta düzey biyofilm üreticisi olduğu saptandı. *C. parapsilosis* kökenlerin biyofilm üretim kapasitelerinin diğer türlerden daha yüksek olduğu gözlemlendi. MİK/2 konsantrasyonda fluoksetin ve sertralin sırasıyla altı ve beş izolatta olgun biyofilm üzerinde inhibe edici etki gösterirken, paroksetin molekülünün yedi izolatın biyofilm oluşumunda artışa yol açtığı saptandı.

TARTIŞMA ve SONUÇ: Bu çalışmanın bulguları, SSRI moleküllerinin sub-MİK konsantrasyonlarda antifungal ve antibiyofilm etkinliklerinin olabileceğini göstermiştir. Antidepresan ilaç molekülleri olan sertralin ve fluoksetinin tek başına ve antifungal ajanlarla kombine kullanımının mantar enfeksiyonlarıyla mücadelede terapötik potansiyeli olabileceği düşünülmektedir.

Anahtar Kelimeler: *Candida* spp., Flukonazol, EUCAST, Sinerjistik etki, antibiyofilm

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INTRODUCTION

Fungal infections have received attention due to their higher prevalence and mortality rates in recent years.¹ Among clinically important yeasts, *Candida* spp. are some of the most common opportunistic pathogens. Although members of this genus may be live as a member of microbiota for healthy individuals, they may cause life-threatening infections in hospitalized and immunosuppressed patients.^{2,3} One of the major reasons causes increase in *Candida* infections, is thought to be the raised usage of medical devices such as catheters, cardiac pacemakers, or artificial heart which have suitable surfaces for biofilm formation.⁴ Biofilm is

a group of microbial cells embedded in extracellular polymeric substances, and recent studies have shown that these sessile cells in biofilms are much more resistant against both antimicrobials and host defense mechanisms compared to planktonic cells due to reduced penetration.⁵

The increased resistance rates to antifungals, high biofilm production capacities and the fact that certain *Candida* species are inherently resistant to some of the antifungals, suggest that new antifungal molecules are needed for therapy. Because of eukaryotic cell structures of fungal pathogens, antifungals should have selective mechanism that target specific structure in microorganism different from human cells. This situation makes it difficult to develop new antifungal agents. Consequently, it is becoming more and more beneficial to investigate the antifungal and antibiofilm activities of various molecules which are used for diverse therapeutic purposes.

Selective serotonin reuptake inhibitors (SSRIs) are being used as antidepressants and as the first-line therapy for premenstrual syndrome. Antifungal activities of these agents were first discovered when three patients with chronic vulvovaginal candidiasis (VVC) were treated with sertraline for premenstrual syndrome, presented no symptoms of candidiasis during the treatment course.⁶ Based on this knowledge, different researches conducted have shown that these agents may have antifungal effects on yeast species. The main goal of this study was to determine the antimicrobial activity and antibiofilm effects of SSRIs alone and in combination with fluconazole, against clinical *Candida* spp isolates.

MATERIALS AND METHODS

Fungal isolates and identification

Twenty *Candida* spp. isolated from the patients samples at Ege University Hospital, Mycology Laboratory of Medical Microbiology Department and *Candida parapsilosis* ATCC 22019 strain were examined. The identification of yeasts species was made by Dalmau method and matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI TOF-MS).

Agent solutions

Fluconazole (Sigma Chemical, USA), fluoxetine (Abdi Ibrahim, Turkey), paroxetine (ARIS, Turkey), and sertraline (Sanovel, Turkey) were provided in powder form. The agents were dissolved with using sterile water and dimethylsulfoxide (DMSO) to a final concentration of 4096 µg/mL. The stock solutions were stored at -80°C until use.

Determination of minimum inhibitory concentrations

Minimum inhibitory concentrations (MICs) of SSRIs and FLC were determined by broth microdilution method according to EUCAST criteria.⁷ Firstly, an appropriate volume of RPMI-1640 (Sigma Chemical, USA) supplemented with 2% glucose (Sigma Chemical, USA) was buffered with 0.165 M MOPS (Sigma Chemical, USA) at pH 7.0. then the medium added to 96-well U-bottom microplates. The agent solutions were added the first well of microplate and serially diluted. Fungal inoculums (1×10^6 cells) were added to the wells and the microplates were incubated at 37°C for 24 h. After the incubation period, the absorbance values were measured at 570 nm by spectrophotometric microplate reader (Varioskan Flash, ThermoScientific, USA). The drug concentration which led to approximately 50% reduction of the growth relative to drug-free well, was accepted as MIC. All experiments were performed in triplicate. The statistical analyzes were performed using GraphPad Prism 5.03 (t-test) program.

Checkerboard assays

Interactions types between SSRI agents and FLC were determined using the checkerboard method in 96-well plates. The types of interaction between SSRI agents and FLC were evaluated based on the fractional inhibitory index (FIX) and the fractional inhibitory concentration (FIC) values for each combination. The following formulas were used to calculate the FIC index.

FIC of drug A = (MIC of drug A in combination) / (MIC of drug A alone)

FIC index (FIX) = (FIC of drug A) + (FIC of drug B.)

Synergistic, indifferent, and antagonist interactions were defined by FIX values of <0.5, 0.5 to 4, and >4, respectively.⁸

Biofilm formation and quantification

Biofilm formation was also quantified by a modification of the crystal violet (CV) staining assay.⁹ Briefly, 100 µl of standardized *Candida* spp. cell suspensions prepared in tryptic soy broth medium (TSB) (Oxoid, UK) (1×10^6 cells) were transferred into wells of a sterile, flat-bottomed, polystyrene 96-well microplates. The microplates were incubated at 37°C for 24 h for biofilm production. Following the incubation period, cell suspensions were aspirated and the wells were washed three times with sterile phosphate buffered saline (PBS-Oxoid, UK) 200 µl per well in order to remove non-adherent cells. After each washing step, the microplates were air dried to remove PBS. Afterwards, the remaining attached microorganisms were fixed with 200 µl of methanol for 15 min. The contents of wells were poured off, methanol was discarded, and the wells were air-dried. Then, 200 µl of 0.02% crystal violet solution was added to the wells for 20 min, at room temperature. After 20 minutes, the CV solution was removed by washing with PBS and the microplates were dried. Each well was destained with 200 µl of 95% ethanol for 15 min. Biofilm formation was quantified by measuring the optical density (O.D.) at 570 nm using a microplate reader (Varioskan Flash, Thermo Scientific, USA). O.D. values of wells without inoculum were used as negative controls. *Enterococcus faecalis* ATCC 29212 was used as positive control strain. The cut-off O.D. (O.D.c) was defined as three standard deviations above the mean O.D. of negative controls. Biofilm production capacities of the isolates were evaluated as shown in Table 1. All tests were carried out in triplicate. The statistical analyzes were performed using GraphPad Prism 5.03 (t-test) program.

Antibiofilm effects of SSRIs

The antibiofilm effects of SSRI agents at sub-MICs (MIC/2, MIC/4), were investigated by crystal violet staining assay. Biofilms formation was performed by adding standardized cell suspensions into wells of the microplates and incubating them for 24 h at 37°C as described above. After biofilm formation, the medium in wells were aspirated, and non-adherent cells were removed by thoroughly washing the all wells three times with sterile PBS. The SSRI agent solutions at sub-MICs (MIC/2 and MIC/4), were prepared in TSB and added to wells which consist preformed biofilm. After adding these agents to wells, the microplates incubated for a further 24 h at 37°C. Then, CV staining assay was performed above. Antibiofilm effects of agents were evaluated by measuring the optical density (O.D.) of wells at 570 nm using a microplate reader.

RESULTS

Fungal isolates and identification

Among twenty clinical fungal isolates were identified six *C. albicans*, four *C. tropicalis*, four *C. krusei*, three *C. parapsilosis* and three *C. glabrata* according to Dalmau method and MALDITOF-MS.

Minimum inhibitory concentrations of fluconazole and SSRIs

Two isolates were found resistant to fluconazole except inherently resistant *C. krusei* isolates. The MICs of sertraline was ranged from 16 µg/mL to 128 µg/mL by the broth microdilution

method while the MICs of paroxetine and fluoxetine were ranged from 64 µg/mL to 512 µg/mL. The MICs of all agents were shown in Table 2.

Checkerboard assay

The interactions between SSRI agents and fluconazole were examined by checkerboard assay. No antagonism was found between tested agents. Fluoxetine showed synergistic effect in the large number of isolates when it compared to other SSRIs. It was also determined that fluoxetine is the only agent showing synergistic interaction with fluconazole against five different *Candida* species. According to checkerboard assay; sertraline, fluoxetine and paroxetine were found synergistic in six, thirteen and ten isolates, respectively. The interaction types of SSRI agents were shown in Table 3.

Biofilm formation and quantification

The biofilm quantification assays revealed that seven of the isolates have weak biofilm production capacity, nine isolates showed moderate biofilm production and four isolates have strong biofilm production capacity. The biofilm production capacities and the number of isolates were shown in Table 4.

Antibiofilm effects of SSRIs

In the presence of MIC/2 of fluoxetine, it was determined that biofilm formation decreased in six isolates while it increased in two isolates. Paroxetine and sertraline, at MIC/2, inhibited biofilm in three and five isolates respectively. The effects of sub-MIC of SSRIs on mature biofilm formation in moderate and strong biofilm producer isolates are shown in Table 5.

DISCUSSION

The significant increase in fungal infections over the past decade has increased the need for new antifungal agents and also reliable and reproducible susceptibility testing methods.¹⁰ There are two reference *in vitro* antifungal susceptibility testing (AFST) methods for *Candida* spp. These reference methods have been developed by two scientific organizations, namely the Clinical and Laboratory Standards Institute (CLSI) and European Committee for Antimicrobial Susceptibility Testing (EUCAST). Despite the differences such as mediums, plate types and measurement methods between these methods, it is determined in several studies that these two methods give consistent results with each other.¹⁰ Although EUCAST method requires more material and equipment, it has a significant advantage to produce results after a 24-hour incubation. Moreover, the measurement of absorbance by the automated device in EUCAST method, instead of visual inspection, that will be major factor that reduce the error rate. Considering these reasons, we first investigated the *in vitro* activity of SSRIs and fluconazole by broth microdilution method according to EUCAST. The agent concentration that led approximately 50% inhibition of the growth relative to the controls, which was determined spectrophotometrically, were accepted the MIC value. (Table 2). Sertraline was the prominent molecule with lower MIC range (16-128 mg/mL) compared to fluoxetine and paroxetine. According to literature, sertraline is generally found to be more effective than others, which is consistent with our study. In a study conducted on *Candida* spp., it had been determined that sertraline has antifungal effects on *Candida* specie and it had also reported that sertraline inhibits *Candida* virulence factors.⁶ The inhibitory effects of sertraline on different yeasts species, such as *Cryptococcus* isolates is also shown by the research.¹¹

There are studies showing that fluoxetine had antibiofilm activity at previously reported MIC values and even at sub-MIC values in literature.¹² In the study conducted by Oliveira Ana S. et al., it was reported that fluoxetine was able to reduce biofilm metabolism at high concentrations, by 96% (*C. krusei*) and biofilm biomass by 82% (*C. glabrata*), when compared to the control. They also detected that sertraline achieved a reduction of 88% on biofilm biomass (*C. glabrata*) and 90% on biofilm metabolism (*C. parapsilosis*), at similar conditions.¹² According to our results, fluoxetine, at sub-MIC concentrations, showed

antibiofilm effect in six isolates, while sertraline showed antibiofilm effect in five isolates. It was also an interesting point that fluoxetine MIC ranges were lower on *C. krusei* isolates compared to other *Candida* species.

Unlike sertraline and fluoxetine, the number of studies about antifungal effects of paroxetine is very limited in literature. However, the results of a study conducted by Costa Silva et al. and our data have shown that paroxetine has antifungal activity at high concentrations.¹³ In parallel to this data we detected the MICs of PRX were higher than FLX and PRX in our study. Considering the our results on antibiofilm effects of PRX, it was noteworthy that PRX, at MIC/2 levels, cause increase in biofilm formation of seven isolates.

Even though it is not fully understood how SSRI agents provide their antifungal activities, the point of interest is that their antifungal activity is independent of the species and resistance properties of the *Candida* isolates. In a study investigating this situation, it was reported that the lethal effect of the agents is related to the induction of apoptosis due to damage to the plasma and mitochondrial membranes. It is thought that this condition may be related to genetic variation rather than factors such as species and resistance patterns.¹³ Although antifungal activities of SSRIs have been shown with many researches in literature, it is necessary to know more about pharmacokinetics of these molecules which usually taken orally in clinical practice. It should investigate that the optimum concentrations that will be reached for these agents in several infection sites with new studies. Considering the plasma drug concentration of SSRIs, it appears that the doses required for *Candida* inhibition are above the commonly using doses of the drugs.^{14, 15} On the other hand, it should be kept in mind that the commonly used dosage regimens and pharmacokinetic data of these drugs are regulated for oral therapeutic use. Undoubtedly, more researches are needed to evaluate using of different forms such as topical formulations of SSRIs as an antimicrobial agent.

Correlation between biofilm formation and antimicrobial resistance profiles was already shown in different studies and so the antibiofilm activities of drug molecules that have known various therapeutic effects are also gaining importance.^{16,17} Therefore, we have also analyzed the antibiofilm effects of SSRI molecules against mature biofilm of *Candida* isolates. Several different methods and devices could be used for detection of biofilm formation such as crystal violet staining assay, light and fluorescence microscopy, bioluminescence, Kongo-Red agar and Christensen methods. Crystal violet staining assay has been used in our study, especially because more sensitive, specific and quantitative results can be obtained by this method.^{18,19} It has been demonstrated with the results of many studies that all *Candida* species could have biofilm forming ability.²⁰ In parallel with these data, the isolates in this study identified as different *Candida* species showed moderate and strong biofilm production capacity (Table 4). It is thought that *C. parapsilosis* have the highest biofilm production capacity among non-*albicans Candida* species when considering the results of both previous reports and our study.^{20,21}

CONCLUSION

It has been understood SSRI agents show in vitro antifungal and antibiofilm activity against *Candida albicans*, *C. tropicalis*, *C. parapsilosis* and *C. glabrata* strains at different concentration levels, thanks to our findings and other researches in literature. In addition to antifungal activity of SSRIs, it was also detected that these agents in combination with fluconazole, could have synergistic against *Candida* spp. The effects of SSRIs on mature biofilms were investigated in this study and it has been found that sertraline and fluoxetine molecules could have antibiofilm effects against *Candida* species. Given all these results and studies, it is thought that these agents could have a potential as adjuvant therapeutic agent. The researches that will be conducted on antibiofilm activities of SSRIs can be beneficial for developmet new antifungal and antibiofilm drug combinations and understanding the mechanisms of their antifungal effects.

Conflict of Interest

No conflict of interest was declared by the authors.

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TABLES

Table 1: Categorizations of biofilm production capacities

Table 2: Minimum inhibitory concentrations of fluconazole and SSRIs

Table 3: Interaction types between SSRIs and fluconazole (FIX values)

Table 4: Biofilm production capacities of the isolates

Table 5: The effects of SSRIs on mature biofilm formation of the isolates

Table 2: Categorizations of biofilm production capacities

$O.D. \leq O.D.c$	No biofilm production
$O.D.c < O.D. \leq (2 \times O.D.c)$	Weak biofilm producer
$(2 \times O.D.c) < O.D. \leq (4 \times O.D.c)$	Moderate biofilm producer
$(4 \times O.D.c) < O.D.$	Strong biofilm producer

O.D: Optical density of the isolate, **O.D.c:** the mean O.D. of negative controls

Table 2: Minimum inhibitory concentrations of fluconazole and SSRIs

Isolate	FLC ($\mu\text{g/mL}$)	SRT ($\mu\text{g/mL}$)	PRX ($\mu\text{g/mL}$)	FLU ($\mu\text{g/mL}$)
<i>Candida glabrata</i>	16	128	256	512
<i>Candida glabrata</i>	16	128	256	512
<i>Candida glabrata</i>	16	128	256	512
<i>Candida albicans</i>	0,25	128	256	512
<i>Candida albicans</i>	0,25	128	256	512
<i>Candida albicans</i>	0,25	128	256	256
<i>Candida albicans</i>	2	64	256	256
<i>Candida albicans</i>	1	64	256	256
<i>Candida albicans</i>	8	64	256	256
<i>Candida tropicalis</i>	1	32	128	128

<i>Candida tropicalis</i>	0,5	32	128	128
<i>Candida tropicalis</i>	4	32	128	256
<i>Candida tropicalis</i>	0,5	32	128	128
<i>Candida krusei</i> *	-	64	64	128
<i>Candida krusei</i> *	-	32	64	128
<i>Candida krusei</i> *	-	32	64	64
<i>Candida krusei</i> *	-	16	128	128
<i>Candida parapsilosis</i>	16	64	256	256
<i>Candida parapsilosis</i>	1	32	512	512
<i>Candida parapsilosis</i>	1	32	256	512
<i>Candida parapsilosis</i> ATCC 22019	2	128	256	512

FLC: Fluconazole, **SRT:** Sertraline, **PRX:** Paroxetine, **FLU:** Fluoxetine **MIC:** Minimum Inhibitory Concentration (µg/mL)

*intrinsically resistant to fluconazole

Table 3: Interaction types between SSRIs and fluconazole (FIX values)

Isolate	FLC+FLX		FLC+SRT		FLC+PRX	
	FIX	Profile	FIX	Profile	FIX	Profile
<i>C. glabrata</i>	0,5078	I	0,5156	I	0,2656	S
<i>C. glabrata</i>	0,375	S	0,5156	I	0,2656	S
<i>C. glabrata</i>	0,5	S	0,5156	I	0,2656	S
<i>C. albicans</i>	0,375	S	0,625	I	0,75	I
<i>C. albicans</i>	0,5	S	1,0313	I	0,75	I
<i>C. albicans</i>	0,5	S	1,25	I	0,75	I
<i>C. albicans</i>	0,2813	S	0,3125	S	0,1563	S
<i>C. albicans</i>	0,1406	S	1,5	I	0,75	I
<i>C. albicans</i>	1,0625	I	1	I	1	I
<i>C. tropicalis</i>	0,5	S	1,5	I	0,75	I
<i>C. tropicalis</i>	1,0625	I	2	I	1,5	I
<i>C. tropicalis</i>	0,2656	S	0,375	S	0,5	S
<i>C. tropicalis</i>	1,0625	I	2	I	1	I
<i>C. krusei</i> *	0,625	I	0,25	S	0,5	S
<i>C. krusei</i> *	0,5	S	0,5	S	0,375	S
<i>C. krusei</i> *	0,5	S	0,5	S	0,375	S
<i>C. krusei</i> *	0,2813	S	0,75	I	0,25	S
<i>C. parapsilosis</i>	0,3125	S	0,25	S	0,2813	S
<i>C. parapsilosis</i>	1,0078	I	1,125	I	1,0078	I
<i>C. parapsilosis</i>	1,0156	I	1,25	I	1,0156	I
<i>C. parapsilosis</i> ATCC 22019	0,5	S	0,2656	S	0,625	I

FLC: Fluconazole, **FLX:** Fluoxetine, **PRX:** Paroxetine, **SRT:** Sertraline,

FIX: Fractional inhibitory index.

*intrinsically resistant to fluconazole.

Table 4: Biofilm production capacities of the isolates

<i>Candida</i> spp.	Biofilm production capacity		
	Weak	Moderate	Strong
<i>C. albicans</i> (n=6)	3	3	-
<i>C. parapsilosis</i> (n=3)	-	-	3
<i>C. krusei</i> (n=4)	2	2	-
<i>C. tropicalis</i> (n=4)	1	2	1
<i>C. glabrata</i> (n=3)	1	2	-

Table 5: The effects of SSRIs on mature biofilm formation of the isolates

Effects on mature biofilm	Number of isolates					
	FLX (MIC/2)	FLX (MIC/4)	PRX (MIC/2)	PRX (MIC/4)	SRT (MIC/2)	SRT (MIC/4)
Decrease	6	4	3	-	5	3
Increase	2	4	7	7	3	3
No effect	5	5	3	6	5	7

FLX: Fluoxetine, **PRX:** Paroxetine, **SRT:** Sertraline,

MIC: Minimum inhibitory concentration

Uncorrected proof