SUPPLEMENTARY

In vitro antimicrobial activity

Microbial strains

Staphylococcus aureus ATCC6538, *Escherichia coli* ATCC25922 and *Candida albicans* ATCC10231 were obtained from American Type Culture Collection.

Qualitative assessment for antimicrobial activity (McFarland 1907; Al-Hiari et al., 2007)

Antimicrobial Susceptibility Test (AST)

Agar well diffusion test was used to determine antimicrobial susceptibility test. In this test, the compound diffuses into the agar medium where the tested microorganism is grown to provide a qualitative assessment for the antimicrobial activity. The compounds were dissolved in DMSO to obtain a final concentration of 10 mg/mL (e.g. A1 6.1 mg dissolved in 0.610 mL DMSO) and so on. An isolated single bacterial colony of an overnight culture grown on TSA was transferred to TSB tube and vortexed to obtain a homogenous suspension. The turbidity was adjusted to 0.5 McFarland standard 1.5 x 10^8 CFU/mL (McFarland 1907;Al-Hiari et al. 2007). A sterile swab was dipped in the tube and squeezed on the wall of the tube to remove excess fluid. Then the swab was streaked on TSA plate in parallel overlapping lines to make a lawn. The plate was left to dry for few minutes. Sterile cork borer was used to punch holes in the agar with a diameter of (0.5 cm). An 80 µL of each compound was added separately to the wells. The plate was incubated at 35 ± 2 ^{o}C for 18-24 hrs. For *C. albicans*, the same procedure was used except that the medium used was SDA. DMSO alone checked for its antimicrobial activity.

Quantitative assessment for antimicrobial activity

Determination of the minimum inhibitory concentration (MIC) of the tested compounds

The compounds that showed antimicrobial activity were further studied to evaluate their MIC against the selected microorganisms.

Preparation of inoculums

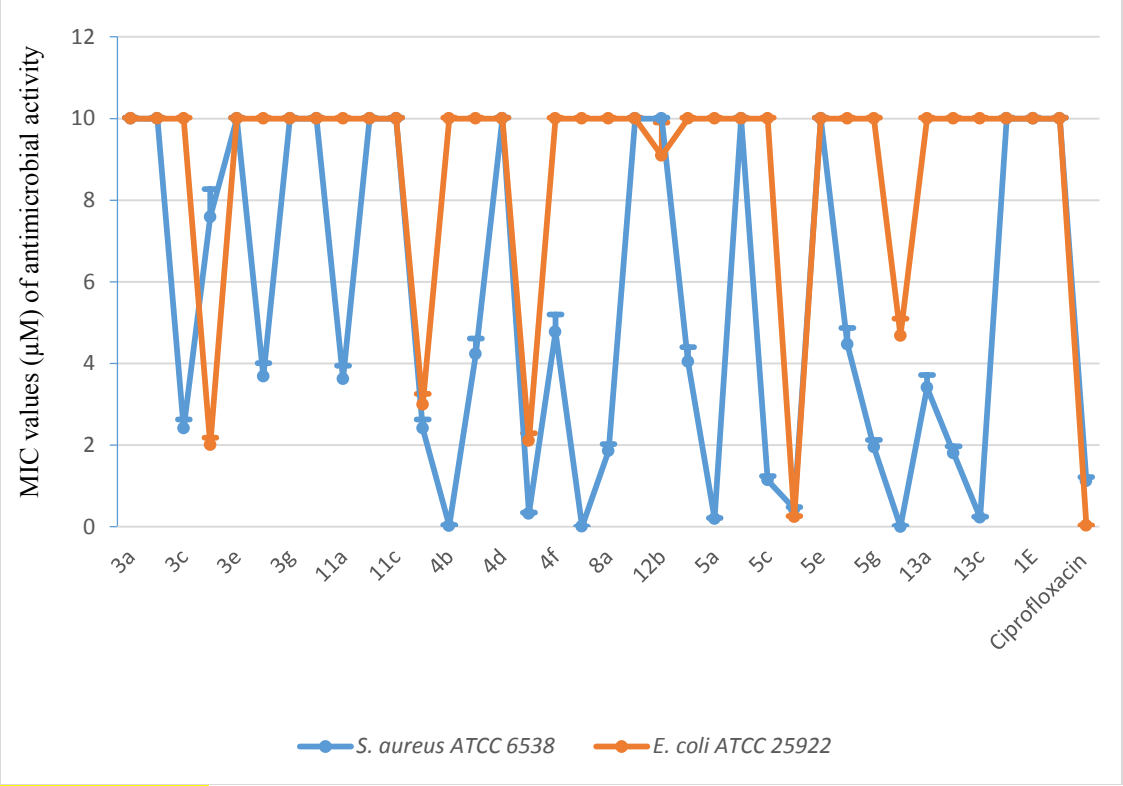
A pure culture of each type of microorganism was inoculated onto TSA, and incubated overnight at 35 ± 2 $^{\circ}$C. A loop full of bacterial culture was aseptically transferred to a sterile tube containing TSB and standardized to 0.5 McFarland, 1.5 x 10^8 CFU/mL. A 10 µL was aseptically transferred to eppendorf tube containing 990 µL of TSB (1/100) to obtain 1.5 x 10^6 CFU/mL.

MIC value determination

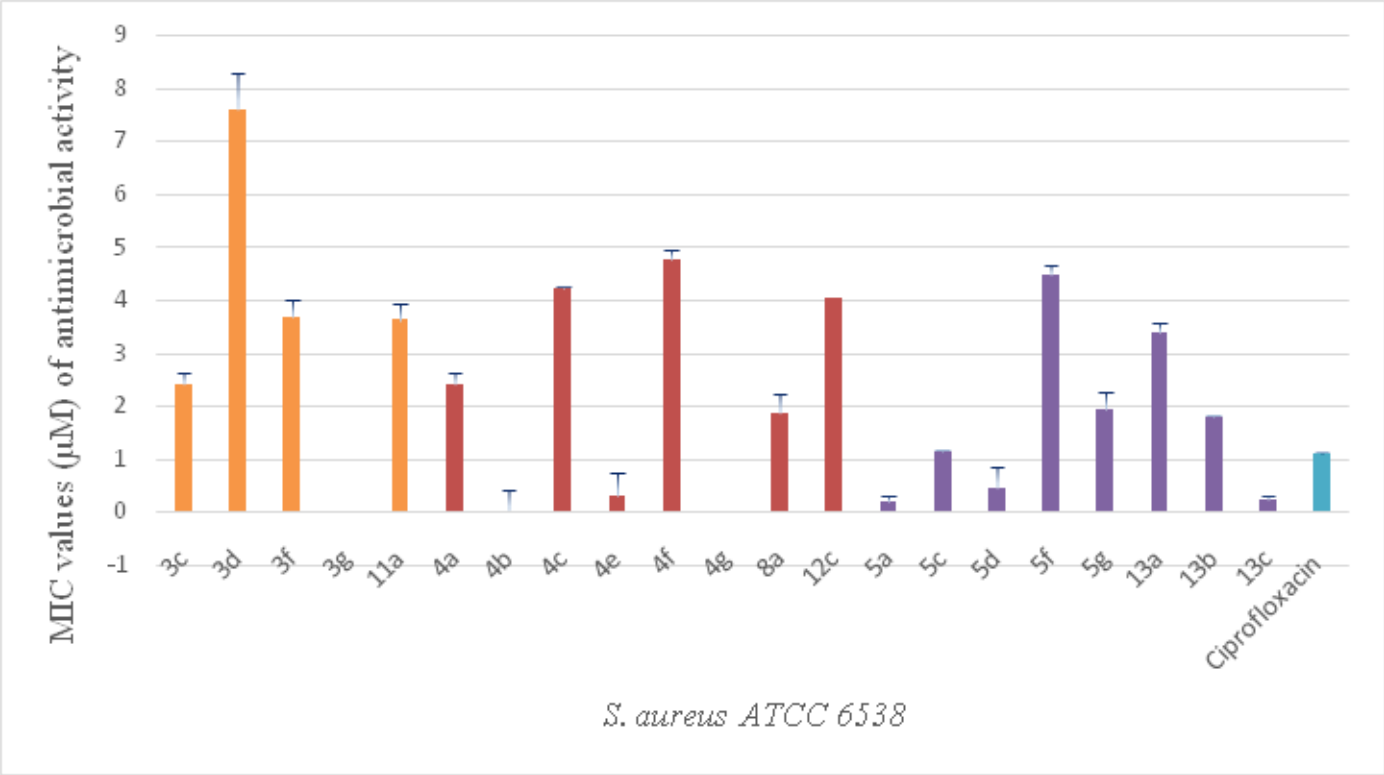
Broth micro dilution method was performed to determine the MIC of the tested compounds. Stock solutions of the compounds were prepared in DMSO. A 200 µL of the tested compounds was transferred to the first row of 96 well plates and 100 µL of TSB was transferred to the other wells. Two-fold serial dilution was performed by transferring 100 µL from the wells of the first row to the second row, mixed and 100 µL was transferred to the third row, mixed, and so on. Then 10 µL from each microbial suspension was inoculated separately into each well except for the first raw. The plates were incubated at 35 ± 2 $^{\circ}$C for 18-24 hr. After incubation the plates were visually inspected for growth (turbidity). Testing of each compound was made in duplicate. A drug -free control was made by inoculating 100 µL of TSB with 10 µL of bacterial suspension. For *C. albicans* testing, the principle of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) method was adopted with modification. In this method TSB was used instead of RPMI medium, since no *C. albicans* growth was obtained using RPMI medium. Two fold serial dilutions of the compounds were prepared as described above.

The wells were inoculated with the *C. albicans* to get a final count of 1.5 x 10^6 CFU/mL. The plates were incubated at 35 ± 2 C° for 18-24 hr. A drug-free control was prepared by inoculating 100 µL of TSB with 10 µL of yeast suspension. After incubation, the absorbance of the wells was measured using a plate reader at 530 nm. MIC is the lowest drug concentration which results in inhibition of growth of ≥50% of that of the drug-free control. Testing of each compound was made in duplicate.

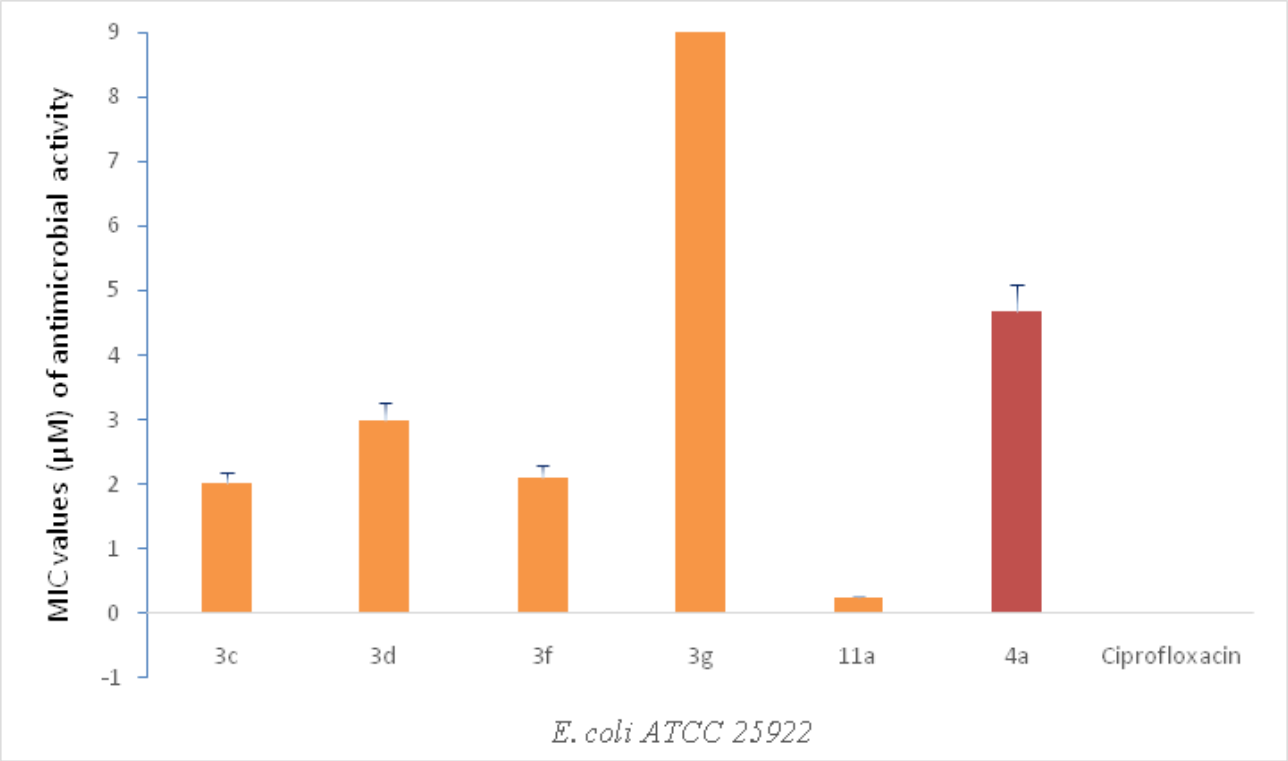
To check for the validity of the analysis, ciprofloxacin HCl antibiotic was used to test for *S. aureus* and *E. coli.* Fluconazole was used to check for *C. albicans.* To check for the antimicrobial activity of DMSO against the microorganisms used, double serial dilutions of DMSO in TSB was prepared as described above to obtain concentrations of DMSO in the range of 50% to 0.3906%. The wells were then inoculated with 10 µL of each of the microbial cultures. From this experiment it was found that DMSO can inhibit microbial growth at concentrations above 12.5%. Therefore, when determining the MIC of the compounds, any result obtained where the concentration of DMSO in it equals or exceeds 12.5% was neglected, because the antimicrobial activity obtained could be attributed to DMSO rather than the compound. The compounds were dissolved in DMSO into the final concentration of 10 mg/mL (e.g. 6.1 mg of A1 dissolved in 0.610 mL of DMSO) and so on.



Supplementary Figure 1. Antibacterial activity of FQs **3-13** (MIC µM values) of both *S.aureus* (blue) and *E.coli* (orange) against ciprofloxacin (below 10 µM) where the cutoff value was 10 µM.



Supplementary Figure 2. Antibacterial activity of FQs with MIC value below 10 µM against *S.aureus* vs. ciprofloxacin.



Supplementary Figure 3. Antibacterial activity of FQs with MIC values below 10 µM against *E.coli* versus ciprofloxacin.

Supplementary 1

Qualitative assessment for antimicrobial activity for *Staphylococcus aureus ATCC 6538*

Code	Compound	ZOI in cm	Code	Compound	ZOI in cm	Code	Compound	ZOI in cm
3a	2-Anis CA	1	4a	R-2-Anis CA	2	5a	T-2-Anis CA	1
3b	3-Anis CA	1.4	4b	R-3-Anis CA	1.1	5b	T-3-Anis CA	1.4
3c	4-Anic CA	1.3	4c	R-4-Anic CA	1.8	5c	T-4-Anic CA	1.7
3d	4-EtACA	1.5	4d	R-4-EtACA	1.5	5d	T-4-EtACA	1.6
3e	4-BuACA	0.8	4e	R-4-BuACA	1	5e	T-4-BuACA	1.1
3f	4-HxACA	0.6	4f	R-4-HxACA	1.5	5f	T-4-HxACA	1.4
3g	2,4-DMeOACA	0.8	4g	R-2,4-DMeOACA	NI	5g	T-2,4-DMeOACA	1.1
7a	CHxCA	1.1	8a	R-CHxCA	1.2	9a	T-CHxCA	1.5
11a	2-Anis CEtA	1.2	12a	R-2-Anis CEtA	1.3	13a	T-2-Anis CEtA	1.5
11b	3-Anis CEtA	0.7	12b	R-3-Anis CEtA	1.6	13b	T-3-Anis CEtA	1.6
11c	2,4-DMeOACETA	1	12c	R-2,4-DMeOACETA	1.5	13c	T-2,4-DMeOACETA	1.6
A1	Cipro Acid “CA”	0.4	A2	Cipro Ester	NI	A3	PF Anilin “CE”	1.7

ZOI: zone of inhibition; NI: No inhibition zone

Supplementary 2

Qualitative assessment for antimicrobial activity for *Escherichia coli ATCC 25922*

Code	Compound	ZOI in cm	Code	Compound	ZOI in cm	Code	Compound	ZOI in cm
3a	2-Anis CA	NI	4a	R-2-Anis CA	2.9	5a	T-2-Anis CA	NI
3b	3-Anis CA	NI	4b	R-3-Anis CA	1.6	5b	T-3-Anis CA	1.4
3c	4-Anic CA	1.9	4c	R-4-Anic CA	3	5c	T-4-Anic CA	1.8
3d	4-EtACA	1.8	4d	R-4-EtACA	2.2	5d	T-4-EtACA	1.2
3e	4-BuACA	NI	4e	R-4-BuACA	1.5	5e	T-4-BuACA	NI
3f	4-HxACA	NI	4f	R-4-HxACA	1.4	5f	T-4-HxACA	NI
3g	2,4-DMeOACA	1.3	4g	R-2,4-DMeOACA	NI	5g	T-2,4-DMeOACA	NI
7a	CHxCA	NI	8a	R-CHxCA	1.4	9a	T-CHxCA	1.1
11a	2-Anis CEtA	1.1	12a	R-2-Anis CEtA	NI	13a	T-2-Anis CEtA	0.9
11b	3-Anis CEtA	NI	12b	R-3-Anis CEtA	1.9	13b	T-3-Anis CEtA	1.9
11c	2,4-DMeOACETA	NI	12c	R-2,4-DMeOACETA	1.6	13c	T-2,4-DMeOACETA	NI
A1	Cipro Acid “CA”	2.2	A2	Cipro Ester	NI	A3	PF Anilin “CE”	NI

ZOI: zone of inhibition; NI: No inhibition zone

Supplementary 3

Qualitative assessment for FQs antimitotic activity against *Candida albicans ATCC 10231*

Code	Compound	ZOI in cm	Code	Compound	ZOI in cm	Code	Compound	ZOI in cm
3a	2-Anis CA	0.7	4a	R-2-Anis CA	0.6	5a	T-2-Anis CA	NI
3b	3-Anis CA	0.5	4b	R-3-Anis CA	0.6	5b	T-3-Anis CA	0.6
3c	4-Anic CA	0.5	4c	R-4-Anic CA	0.5	5c	T-4-Anic CA	0.9
3d	4-EtACA	0.8	4d	R-4-EtACA	NI	5d	T-4-EtACA	0.6
3e	4-BuACA	0.7	4e	R-4-BuACA	0.7	5e	T-4-BuACA	0.8
3f	4-HxACA	NI	4f	R-4-HxACA	0.9	5f	T-4-HxACA	0.8
3g	2,4-DMeOACA	0.8	4g	R-2,4-DMeOACA	0.5	5g	T-2,4-DMeOACA	0.7
7a	CHxCA	1.3	8a	R-CHxCA	0.7	9a	T-CHxCA	0.5
11a	2-Anis CEtA	0.6	12a	R-2-Anis CEtA	0.9	13a	T-2-Anis CEtA	0.6
11b	3-Anis CEtA	NI	12b	R-3-Anis CEtA	NI	13b	T-3-Anis CEtA	NI
11c	2,4-DMeOACETA	0.7	12c	R-2,4-DMeOACETA	NI	13c	T-2,4-DMeOACETA	0.7
A1	Cipro Acid “CA”	1.4	A2	Cipro Ester	0.5	A3	PF Anilin “CE”	0.7

ZOI: zone of inhibition; NI: No inhibition zone

Supplementary 4

Minimum inhibitory concentrations of the compounds against *S. aureus ATCC 6538*

Code	Compound	MIC in µM	Code	Compound	MIC in µM	Code	Compound	MIC in µM
3a	2-Anis CA	19.95 ± 1.8**	4a	R-2-Anis CA	2.41 ± 0.22**	5a	T-2-Anis CA	0.2 ± 0.02**
3b	3-Anis CA	15.11 ± 1.36**	4b	R-3-Anis CA	0.024 ± 0.00^{ns}	5b	T-3-Anis CA	33.04 ± 2.97**
3c	4-Anic CA	2.41 ± 0.22**	4c	R-4-Anic CA	4.23 ± 0.38**	5c	T-4-Anic CA	1.14 ± 0.10**
3d	4-EtACA	7.59 ± 0.68**	4d	R-4-EtACA	50.28 ± 4.53**	5d	T-4-EtACA	0.44 ± 0.04**
3e	4-BuACA	28.44 ± 2.56**	4e	R-4-BuACA	0.32 ± 0.03**	5e	T-4-BuACA	30.99 ± 2.79**
3f	4-HxACA	3.68 ± 0.33**	4f	R-4-HxACA	4.77 ± 0.429**	5f	T-4-HxACA	4.47 ± 0.40**
3g	2,4-DMeOACA	NI	4g	R-2,4-DMeOACA	0.004 ± 0.00**	5g	T-2,4-DMeOACA	1.95 ± 0.16**
7a	CHxCA	42.37 ± 3.81**	8a	R-CHxCA	1.86 ± 0.17**	9a	T-CHxCA	44.66 ± 4.02**
11a	2-Anis CEtA	3.62 ± 0.36**	12a	R-2-Anis CEtA	26.38 ± 2.37**	13a	T-2-Anis CEtA	3.408 ± 0.31**
11b	3-Anis CEtA	10.38 ± 0.93**	12b	R-3-Anis CEtA	11.202 ± 1.01**	13b	T-3-Anis CEtA	1.804 ± 0.16**
11c	2,4-DMeOACETA	38.71 ± 3.48**	12c	R-2,4-DMeOACETA	4.04 ± 0.36**	13c	T-2,4-DMeOACETA	0.22 ± 0.02**
A1	Cipro Acid “CA”	19.89 ± 1.79**	A2	Cipro Ester	NI	A3	PF Anilin “CE”	NI
Ref.	Ciprofloxacin	1.12 ± 0.10						

Results are mean ± SD, P-value calculated by unpaired t-test between test compound IC_{50} values and ciprofloxacin (µM) using Graph Pad Prism software version 5.0.1.* when *P*<0.05 and ** when *P*< 0.01 or 0.001, *** when P<0.0001, NS: not significantly different from reference agent. NI: no Inhibition zone. MIC: Minimum inhibitory concentration. SD: Standard deviation.

Supplementary 5

Minimum inhibitory concentration of the compounds against *E. coli ATCC 25922*

Code	Compound	MIC in µM	Code	Compound	MIC in µM	Code	Compound	MIC in µM
3a	2-Anis CA	NI	4a	R-2-Anis CA	2.99 ± 0.27**	5a	T-2-Anis CA	NI
3b	3-Anis CA	NI	4b	R-3-Anis CA	169.54 ± 15.26**	5b	T-3-Anis CA	99.14 ± 8.93**
3c	4-Anic CA	50.31 ± 4.53**	4c	R-4-Anic CA	NI	5c	T-4-Anic CA	39.27 ± 3.53**
3d	4-EtACA	2.01 ± 0.18**	4d	R-4-EtACA	47.56 ± 4.28**	5d	T-4-EtACA	0.239 ± 0.02**
3e	4-BuACA	NI	4e	R-4-BuACA	2.105 ± 0.19**	5e	T-4-BuACA	NI
3f	4-HxACA	NI	4f	R-4-HxACA	10.55 ± 0.95**	5f	T-4-HxACA	NI
3g	2,4-DMeOACA	NI	4g	R-2,4-DMeOACA	53.21 ± 4.79**	5g	T-2,4-DMeOACA	NI
7a	CHxCA	NI	8a	R-CHxCA	61.77 ± 5.56**	9a	T-CHxCA	4.68 ± 0.42**
11a	2-Anis CEtA	37.73 ± 3.40**	12a	R-2-Anis CEtA	NI	13a	T-2-Anis CEtA	36.18 ± 3.26**
11b	3-Anis CEtA	NI	12b	R-3-Anis CEtA	9.088 ± 0.82**	13b	T-3-Anis CEtA	12.114 ± 1.09**
11c	2,4-DMeOACETA	NI	12c	R-2,4-DMeOACETA	NI	13c	T-2,4-DMeOACETA	NI
A1	Cipro Acid “CA”	10.622 ± 0.96**	A2	Cipro Ester	NI	A3	PF Anilin “CE”	NI
Ref.	Ciprofloxacin	0.0278 ± 0.00						

Results are mean ± SD, P-value calculated by unpaired t-test between test compound IC_{50} values and ciprofloxacin (µM) using Graph Pad Prism software version 5.0.1.* when $P<0.05$ and ** when $P< 0.01$ or 0.001, *** when $P<0.0001$, NS: not significantly different from reference agent. NI: no Inhibition zone. MIC: Minimum inhibitory concentration. SD: Standard deviation

Supplementary 6

Minimum inhibitory concentration of the compounds against *C. albicans ATCC 10231*

Code	Compound	MIC in µM	Code	Compound	MIC in µM	Code	Compound	MIC in µM
3a	2-Anis CA	No MIC obtained*	4a	R-2-Anis CA	No MIC obtained*	5a	T-2-Anis CA	No MIC obtained*
3b	3-Anis CA	No MIC obtained*	4b	R-3-Anis CA	No MIC obtained*	5b	T-3-Anis CA	No MIC obtained*
3c	4-Anic CA	No MIC obtained*	4c	R-4-Anic CA	No MIC obtained*	5c	T-4-Anic CA	No MIC obtained*
3d	4-EtACA	No MIC obtained*	4d	R-4-EtACA	No MIC obtained*	5d	T-4-EtACA	No MIC obtained*
3e	4-BuACA	No MIC obtained*	4e	R-4-BuACA	76.199± 8.01^{+}	5e	T-4-BuACA	99.29± 10.00^{+}
3f	4-HxACA	No MIC obtained*	4f	R-4-HxACA	No MIC obtained*	5f	T-4-HxACA	No MIC obtained*
3g	2,4-DMeOACA	No MIC obtained*	4g	R-2,4-DMeOACA	No MIC obtained*	5g	T-2,4-DMeOACA	No MIC obtained*
7a	CHxCA	No MIC obtained*	8a	R-CHxCA	230.94±20.22^{+}	9a	T-CHxCA	No MIC obtained*
11a	2-Anis CEtA	No MIC obtained*	12a	R-2-Anis CEtA	No MIC obtained*	13a	T-2-Anis CEtA	No MIC obtained*
11b	3-Anis CEtA	No MIC obtained*	12b	R-3-Anis CEtA	No MIC obtained*	13b	T-3-Anis CEtA	No MIC obtained*
11c	2,4-DMeOACETA	No MIC obtained*	12c	R-2,4-DMeOACETA	No MIC obtained*	13c	T-2,4-DMeOACETA	No MIC obtained*
A1	Cipro Acid “CA”	No MIC obtained*	A2	Cipro Ester	No MIC obtained*	A3	PF Anilin “CE”	No MIC obtained*
Ref.	Fluconazole	9570.96± 861.38						

* Because of solubility issues. Results are mean ± SD, P-value calculated by unpaired t-test between test compound IC_{50} values and fluconazole (µM) using Graph Pad Prism software version 5.0.1. $^{+}$When $P<0.05$, NS: not significantly different from reference agent. NI: no Inhibition zone. MIC: Minimum inhibitory concentration. SD: Standard deviation