# 1- ANTICANCER SCREENING

**Experimental:**

Six compounds **3, 13, 15, 16a, 16b and 18** were selected by the *National cancer institute* (NCI), Bethesda, Maryland, USA to be evaluated for their *in vitro* antitumor activity. The operation of this screen utilizes 60 different human tumor cell lines, representing leukemia, melanoma and cancers of the lung, colon, brain, ovary, breast, prostate, and kidney. This screen is unique in that the complexity of a 60 cell line dose response produced by a given compound results in a biological response pattern which can be utilized in pattern recognition algorithms (COMPARE) program. Using these algorithms, it is possible to assign a putative mechanism of action to a test compound, or to determine that the response pattern is unique and not similar to that of any of the standard prototype compounds included in the NCI database. In addition, following characterization of various cellular molecular targets in the 60 cell lines, it may be possible to select compounds most likely to interact with a specific molecular target.

The screening is a two-stage process, beginning with the evaluation of all compounds against the 60 cell lines at a single dose of 10 µM. The output from the single dose screen is reported as a mean graph and is available for analysis by the COMPARE program. Compounds which satisfy predetermined threshold inhibition criteria based on careful analysis of historical DTP screening data using COMPARE algorithms will proceed to the 5-dose assay.

**Methodology of the *in vitro* cancer screening**

The human tumor cell lines of the cancer screening panel are grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. For a typical screening experiment, cells are inoculated into 96 well microtiter plates in 100 µL at plating densities ranging from 5,000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates are incubated at 37° C, 5 % $CO_2$, 95 % air and 100 % relative humidity for 24 h prior to addition of experimental drugs.

After 24 h, two plates of each cell line are fixed *in situ* with TCA, to represent a measurement of the cell population for each cell line at the time of drug addition (Tz). Experimental drugs are solubilized in DMSO at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate is thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 µg/ml gentamicin. Additional four, 10-fold or ½ log serial dilutions are made to provide a total of five drug concentrations plus control. Aliquots of 100 µl of these different drug dilutions are added to the appropriate microtiter wells already containing 100 µl of medium, resulting in the required final drug concentrations.

Following drug addition, the plates are incubated for an additional 48 h at 37°C, 5 % $CO_2$, 95 % air, and 100 % relative humidity. For adherent cells, the assay is terminated by the addition of cold TCA. Cells are fixed *in situ* by the gentle addition of 50 µl of cold 50 % (w/v) TCA (final concentration, 10 % TCA) and incubated for 60 minutes at 4°C. The supernatant is discarded, and the plates are washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (100 µl) at 0.4 % (w/v) in 1 % acetic acid is added to each well, and plates are incubated for 10 minutes at room temperature. After staining, unbound dye is removed by washing five times with 1 % acetic acid and the plates are air dried. Bound stain is subsequently solubilized with 10 mM trizma base, and the absorbance is read on an automated plate reader at a wavelength of 515 nm. For suspension cells, the methodology is the same except that the assay is terminated by fixing settled cells at the bottom of the wells by gently adding 50 µl of 80 % TCA (final concentration, 16 % TCA). Using the seven absorbance measurements [time zero, (Tz), control growth, (C), and test growth in the presence of drug at the five concentration levels (Ti)], the percentage growth is calculated at each of the drug concentrations levels. Percentage growth inhibition is calculated as:

[(Ti-Tz)/(C-Tz)] x 100 for concentrations for which Ti>/=Tz

[(Ti-Tz)/Tz] x 100 for concentrations for which Ti<Tz.

**Interpretation of One-Dose Data:**

Results for each tested compound were reported as a mean graph of the percent growth of the treated cells when compared to the untreated control cells. This allows detection of both growth inhibition (values between 0 and 100) and lethality (values less than 0).

**Table 12: Renal cancer cell lines which showed highest activity and their growth percentage (G %) in single-dose assay**

| Compound ID | Renal cancer (G %) | |
|---|---|---|
| | **A498** | **UO-31** |
| **3** | 53.28 | 73.54 |
| **13** | 97.47 | 102.03 |
| **15** | 89.25 | 87.02 |
| **16a** | 58.21 | 71.72 |
| **16b** | -32.10 | 87.45 |
| **18** | 63.23 | 75.39 |

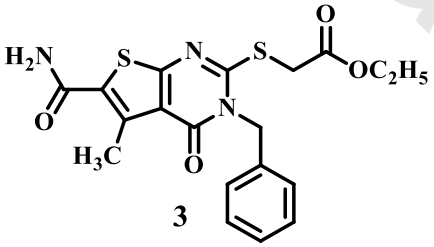
**Results and discussion of anticancer activity**

All the compounds submitted to the NCI, 6 compounds were tested initially at a single dose (10 µM) in the full NCI 60 cell line panel.

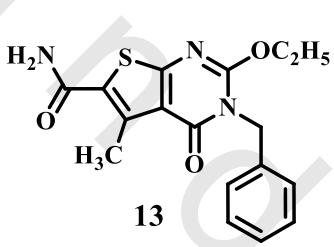
Results were presented as one dose mean graph in **table 12**. The tested compounds showed variable activity against renal cancer A498 and UO-31 cell lines. Compounds **3**, **16a** and **18** showed moderate anticancer activity against renal cancer A498 cell line exhibiting 46.72, 41.79 and 36.77 % growth inhibition, respectively whereas compound **13** and **15** exerted weak growth inhibition of 2.53 and 10.75 %, respectively. On the other hand, compounds **3**, **16a** and **18** showed mild growth inhibition of 26.46, 28.28 and 24.61 % against renal cancer UO-31 cell line while compound **15** and **16b** exerted weak inhibitory effect towards the same cell line with 12.98 and 12.55% growth inhibition, respectively.

Furthermore, compound **16b** showed lethal effect towards renal cancer A498 cell line by 32.10%.

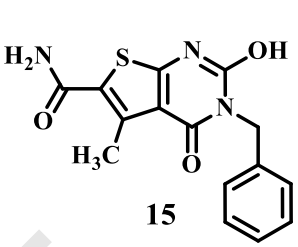
On the other hand, none of the tested compounds satisfied the threshold inhibition criteria and did not pass forward for evaluation in the full panel five-dose *in vitro* antitumor screen.



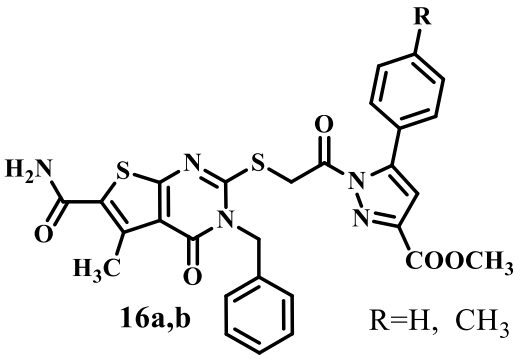
**3**



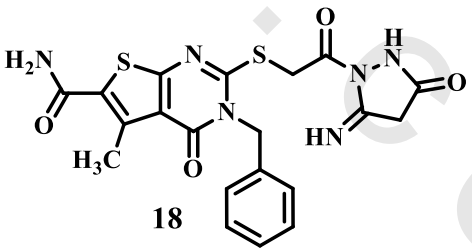
**13**



**15**



**16a,b** R=H, $CH_3$



**18**

The structure activity relationship of the tested compounds indicated that, the moderate anticancer activity displayed by compounds **3**, **16a** and **18** might be attributed to the presence of the O-$C_2H_5$ in compound **3** or the nitrogen heterocyclic rings (pyrazole ring in **16a** and pyrazolidinone ring in **18**) attached to the thienopyrimidine nucleus through three atom spacer. Whereas, elimination of such atom spacer in compounds **13** and **15** resulted in weak anticancer activity.

## 2- ANTIMICROBIAL SCREENING

The antimicrobial screening of the target compounds was performed in *Medical Biotechnology Department, Genetic Engineering and Biotechnology Research Institute (GEBRI), Borg El-Arab, Alexandria, Egypt.*

## Experimental:

Thirty six compounds were preliminarily evaluated for their *in vitro* antibacterial activity against *S. aureus* and *B. subtilis* as representatives of Gram-positive bacteria; *E. coli* and *P. aeruginosa* as representatives of Gram-negative bacteria. The compounds were also evaluated for their *in vitro* antifungal activity against *C. albicans*. Their inhibition zones using the cup diffusion technique [(108)] were measured. Further evaluation was then carried out on the test compounds to determine their minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) using the two-fold serial dilution method.[(109)] Ampicillin was used as standard antibacterial while clotrimazole was used as antifungal reference. Dimethylsulfoxide (DMSO) was used as a blank and showed no antimicrobial activity.

**Inhibition zones measurement**

The compounds were dissolved in DMSO at a concentration of 1 mg/ml. Each 100 ml of sterile molten agar (at 45°C) received 1 ml of 6 h-broth culture and then the seeded agar was poured into sterile Petri-dishes. Cups (8 mm in diameter) were cut in the agar. Each cup received 0.1 ml of the 1 mg/ml solution of the test compounds. The plates were then incubated at 37°C for 24 h or for 48 h for *C. albicans*. A control using DMSO without the test compound was included for each organism. Ampicillin was used as standard antibacterial, while clotrimazole was used as antifungal reference. The resulting inhibition zones are recorded (**Table 13**).

**Minimal inhibitory concentration (MIC) measurement**

The test organisms were grown in their suitable broth for 24 h for bacteria and 48 h for fungi at 37°C. Two fold serial dilutions of solutions of the test compounds were prepared using 200, 100, 50, 25 and 12.5µg/ml. The tubes were then inoculated with the test organisms; each 5 ml received 0.1 ml of the above inoculum and were incubated at 37°C for 48 h. Then, the tubes were observed for the presence or absence of microbial growth. The MIC values of the prepared compounds are listed in **table 13**.

**Minimal bactericidal concentration (MBC) measurement**

MIC tests were always extended to measure the MBC as follows: A loop-full from the tube not showing visible growth (MIC) was spread over a quarter of Müller–Hinton agar plate. After 18 h of incubation, the plates were examined for growth. Again, the tube containing the lowest concentration of the test compound that failed to yield growth on sub-culture plates was judged to contain the MBC of that compound for the respective test organism (**Table 13**).

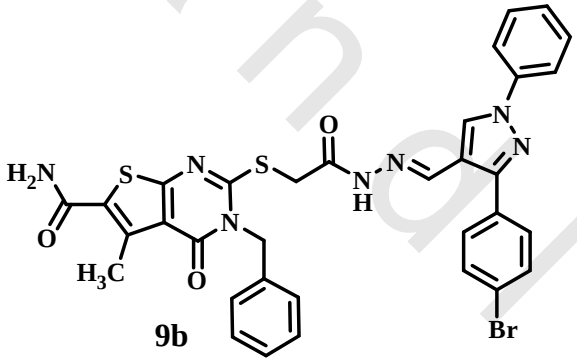
## Results and Discussion:

Inspection of the antimicrobial screening data recorded in **table 13** revealed that twenty nine compounds **(4, 9a-c, 10a, 10b, 10d, 13, 15, 16a,b, 17a, 18, 19, 20b, 21, 23a,b, 24a,b, 25, 26a,b and 27a-c)** out of thirty six exhibited promising antimicrobial activity.

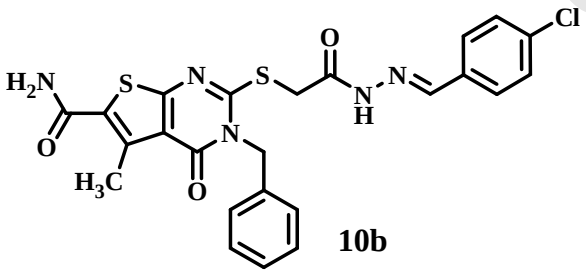
In general, the tested compounds showed distinctive activity against the gram negative bacteria *P. aeruginosa*.

**Antibacterial activity:**

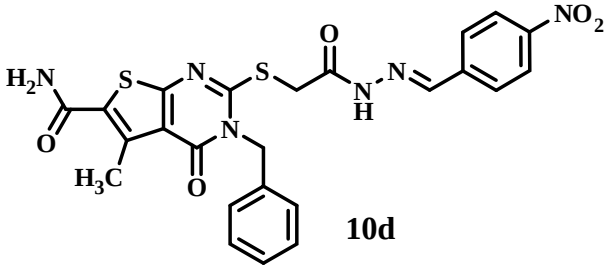
The results illustrated in **table 13** revealed that compounds **9b, 10b, 10d, 17a, 19, 23a,b and 24a,b** exerted considerable activity towards *P. aeruginosa*. They were two times (MIC = 25 µg/ml) as active as ampicillin (MIC = 50 µg/ml). In addition, compounds **4, 9a, 9c, 10a, 13, 15, 16a,b, 18, 20b, 21, 25, 26a,b** and **27a-c,** were as active as the reference.



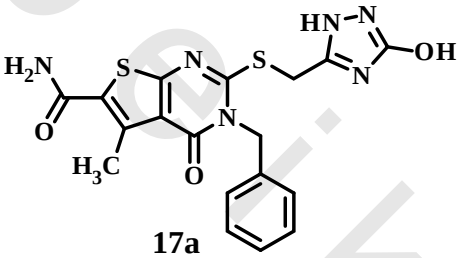
**9b**



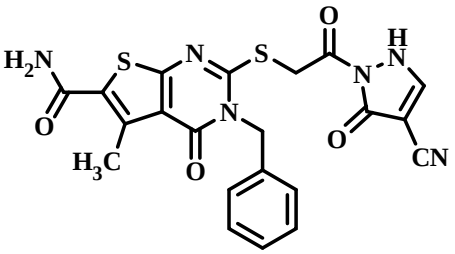
**10b**



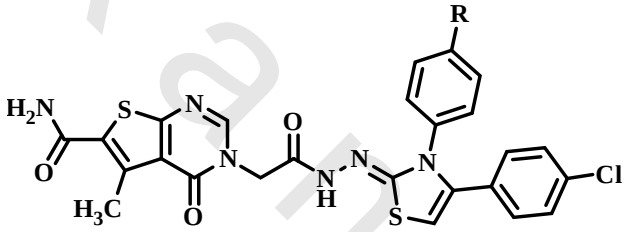
**10d**



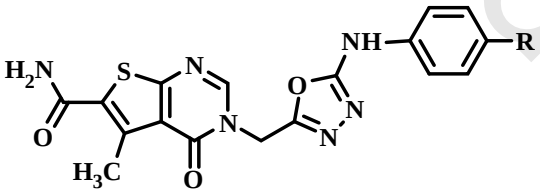
**17a**



**19**



**23a,b**



R= Cl, $OCH_3$

**24a,b**

Furthermore, compounds **4, 16a, 21, 26a** and **27a** showed moderate activity against *E. coli* (MIC = 25 µg/ml) compared to ampicillin (MIC = 10 µg/ml).

On the other hand, compounds **9b, 10a, 10d, 18, 23a, 23b** and **24b** had nearly one half the activity of the reference against *B. subtilis* (MIC = 25 µg/ml).

It is worthy to mention that, compounds **9b, 10d, 23a,b** and **24b** displayed good antimicrobial activity against both *B. subtilis and P.aeruginosa* .

Unfortunately all the tested compounds showed no notable antibacterial activity against *S. aureus.*

**Antifungal activity:**

Compounds **4, 10a, 18** and **21** displayed mild antifungal activity (MIC = 25 µg/ml), However, Compounds **9a,b, 10d, 13, 16a,b, 22a, 23a,b, 24b, 25, 26a,b** and **27a-c** demonstrated weak activity against *C.albicans* (MIC = 50 µg/ml).

According to the MIC and MBC limits derived from the latest National Committee on Clinical Laboratory Standards (NCCLS), we can determine whether the test compound is bactericidal or bacteriostatic to the test organism. If the MBC = MIC, the test compound is considered bactericidal but if MBC > MIC the test compound is considered bacteriostatic. Accordingly, as revealed from table 13, compounds **4, 10a, 16a, 18, 21, 26a** and **27a** were bactericidal against *P. aeruginosa* while other compounds were bacteriostatic.

## Correlation of structure to preliminary antimicrobial screening (MIC data, activity):

Structural activity correlation of the tested compounds indicated that the acid hydrazide **4** exhibited promising antibacterial activity against *P.aeruginosa* comparable to that of ampicillin and showed moderate activity against *E.coli*. In addition, it showed mild antifungal activity against *C.albicans.* Replacement of the 2-hydrazinyl-2-oxoehtyl thio moiety at position-2 of the acid hydrazide **4** by OH or $OC_2H_5$ functions gave compounds **13** and **15**, respectively which were equipotent and displayed the same antimicrobial activity of ampicillin against *P.aeruginosa.* Furthermore, compound **13** showed weak antifungal activity against *C.albicans.* Condensation of the acid hydrazide **4** with pyrazole or aryl aldehydes afforded compounds **9a-c** and **10a-d**, respectively. Among the $N^2$-(3-aryl-1-phenyl-1H-pyrazol-4-yl)methylidene acetohydrazide derivatives **9a-c,** compound **9b** with 4-chlorophenyl moiety at position-3 of the pyrazole ring displayed two times the activity of ampicillin against *P.aeruginosa* with weak antifungal activity against *C.albicans.* While the phenyl and 4-tolyl **9a,b** analogues displayed the same activity of ampicillin against *P.aeruginosa*. In addition compound **9a** showed nearly one half the activity of the reference against *B.subtilis* and weak antifungal activity against *C.albicans.* Among the arylidene acetohydrazide derivatives **10a-d,** compound **10b** and compound **10d** having 4-chlorophenyl or 4-nitrophenyl moieties, respectively were equipotent, showing the highest activity against *P.aeruginosa* displaying two times the ampicillin activity, whereas the unsubstituted analogue **10a** retained the same antimicrobial activity as ampicillin against *P.aeruginosa*. Furthermore, compounds **10a** and **10d** were equipotent against *B.subtilis* displaying nearly one half the activity of ampicillin. In addition, compound **10a** showed mild antifungal activity against *C.albicans*, whereas **10d** displayed weak antifungal activity.

On the other hand, the 4-$OCH_3$ analogue **10c** displayed one half the ampicillin activity against *P.aeruginosa.*

Cyclocondensation of the acid hydrazide **4** afforded the variously substituted pyrazole derivatives **16**, **18** and **19**. Among these compounds, the 4-cyano-5-oxo-2,5-dihydro-1*H*-pyrazol-1-yl derivative **19** displayed two times the ampicillin activity against *P.aeruginosa* while the methyl 5-(substituted phenyl)-*1H*-pyrazole-3-carboxylate derivatives **16a,b** and 5-imino-3-oxopyrazolidine **18** were equipotent against *P.aeruginosa* displaying the same antimicrobial activity of ampicillin. In addition, compound **16a** revealed moderate activity against *E.coli* while compound **18** displayed nearly one half the activity of ampicillin against *B.subtilis.* Furthermore, compound **18** showed mild antifungal activity against *C.albicans*, whereas compounds **16a,b** displayed weak antifungal activity.

Cyclocondensation of **4** with urea or thiourea yielded the corresponding triazole derivatives **17a,b**. The 5-hydroxy triazole derivative **17a** displayed two times the ampicillin activity against *P.aeruginosa*, whereas the 5-mercapto analogue **17b** showed one half the activity of the reference.

Converting the acid hydrazide **4** to the respective 2,5-dioxopyrrolidin-1-ylamino derivative **20a** by cyclocondensation with succinic anhydride decreased the antimicrobial activity as compared with the precursor **4** showing one half the antimicrobial activity of ampicillin. In contrast, the 1,3-dioxoisoindolin-2-ylamino analogue **20b** produced by cyclocondensation with phthalic anhydride retained the same antimicrobial activity of ampicillin against *P.aeruginosa.*

Moreover, the results of antimicrobial screening showed that 3-(2-hydrazinyl-2-oxoethyl)-5-methyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-6-carboxamide **7** displayed one half the antimicrobial activity of ampicillin against *P.aeruginosa.*

Reaction of the acid hydrazide **7** with diethyl ethylmalonate or with the appropriate substituted pyrazole aldehydes increased the antimicrobial activity of the obtained ethyl butanoate derivative **21** and $N^2$-(3-aryl-1-phenyl-*1H*-pyrazol-4-yl)methylidene acetohydrazide derivatives **27a-c** displaying two times the ampicillin activity against *P.aeruginosa.*

Compound **21** and **27a** showed moderate activity against *E.coli.* In addition, compound **21** showed mild antifungal activity against *C.albicans*. Furthermore, compounds **27a-c** showed weak antifungal activity against *C.albicans.*

Converting the acid hydrazide **7** to the open chain *N*-substituted thiosemicarbazide derivatives **22a,b** did not improve the antimicrobial activity that was one half the activity of ampicillin against *P.aeruginosa*. In addition compound **22a** showed weak antifungal activity against *C.albicans.*

Cyclization of these thiosemicarbazides **22a,b** into a rigid 5-membered heterocyclic ring structures afforded the thiazolidine **23a,b** , oxadiazole **24a,b** and thiadiazole **25** derivatives displaying improved antimicrobial activity. The thiazolidine **23a,b** and oxadiazole **24a,b** derivatives were equipotent against *P.aeruginosa* displaying two times the ampicillin activity, whereas the thiadiazole derivative **25** showed the same activity of ampicillin. Furthermore, compounds **23a,b** and **24b** demonstrated one half the activity of ampicillin against *B.subtilis,* while compounds **23a,b** , **24b** and **25** displayed weak antifungal activity against *C.albicans.*

In addition, conversion of the thiosemicarbazides **22a,b** to the corresponding ethyl acetate eater derivatives **26a,b** improved the antimicrobial activity of the precursor, they were equipotent and retained the same antimicrobial activity of ampicillin against *P.aeruginosa.* In addition, compounds **26a,b** showed weak antifungal activity against *C.albicans.* Moreover, **26a** displayed moderate activity against *E.coli.*

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**Table 13: Antimicrobial activities of the tested compounds**

| Comp. No. | ***S. aureus*** (DSM 1104) | | | ***B. subtilis*** (ATCC 6633) | | | ***P. aeruginosa*** (ATCC 10145) | | | ***E. coli*** (ATCC 11775) | | | ***C. albicans*** (DSM 70014) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *IZ* | *MIC* | *MBC* | *IZ* | *MIC* | *MBC* | *IZ* | *MIC* | *MBC* | *IZ* | *MIC* | *MBC* | *IZ* | *MIC* | *MFC* |
| **3** | 15 | 50 | 50 | 16 | 50 | 50 | 18 | 100 | 100 | 16 | 100 | 100 | 14 | 100 | 100 |
| **4** | 16 | 25 | 50 | 14 | 100 | 100 | 14 | 50 | 50 | 16 | 25 | 50 | 15 | 25 | 50 |
| **7** | 16 | 50 | 50 | 16 | 50 | 50 | 14 | 100 | 100 | 18 | 100 | 100 | 17 | 100 | 100 |
| **9a** | 16 | 25 | 50 | 15 | 50 | 50 | 18 | 50 | 100 | 16 | 50 | 100 | 15 | 50 | 100 |
| **9b** | 18 | 50 | 50 | 14 | 25 | 50 | 12 | 25 | 50 | 15 | 50 | 50 | 16 | 50 | 50 |
| **9c** | 15 | 50 | 100 | 16 | 100 | 100 | 13 | 50 | 100 | 14 | 50 | 50 | 17 | 100 | 100 |
| **10a** | 15 | 50 | 50 | 12 | 25 | 50 | 13 | 50 | 50 | 16 | 50 | 50 | 15 | 25 | 50 |
| **10b** | 18 | 100 | 100 | 14 | 50 | 50 | 16 | 25 | 50 | 18 | 100 | 100 | 16 | 100 | 100 |
| **10c** | 14 | 100 | 200 | 16 | 100 | 100 | 12 | 100 | 100 | 17 | 50 | 100 | 18 | 100 | 100 |
| **10d** | 16 | 50 | 50 | 14 | 25 | 50 | 18 | 25 | 50 | 15 | 50 | 50 | 15 | 50 | 50 |
| **11** | 17 | 100 | 200 | 14 | 100 | 100 | 14 | 100 | 100 | 16 | 50 | 100 | 17 | 100 | 100 |
| **12** | 17 | 100 | 200 | 16 | 100 | 100 | 18 | 100 | 100 | 14 | 50 | 100 | 14 | 100 | 100 |
| **13** | 14 | 50 | 50 | 14 | 50 | 50 | 16 | 50 | 100 | 13 | 50 | 100 | 12 | 50 | 50 |
| **14** | 18 | 100 | 200 | 14 | 100 | 100 | 12 | 100 | 100 | 15 | 50 | 100 | 16 | 100 | 100 |
| **15** | 14 | 50 | 100 | 14 | 100 | 100 | 16 | 50 | 100 | 17 | 50 | 50 | 18 | 100 | 100 |
| **16a** | 12 | 25 | 50 | 18 | 50 | 50 | 16 | 50 | 50 | 17 | 25 | 50 | 14 | 50 | 50 |
| **16b** | 14 | 50 | 50 | 18 | 50 | 50 | 13 | 50 | 100 | 14 | 50 | 100 | 17 | 50 | 50 |
| **17a** | 12 | 100 | 100 | 16 | 50 | 50 | 14 | 25 | 50 | 15 | 100 | 100 | 14 | 100 | 100 |
| **17b** | 14 | 100 | 200 | 12 | 100 | 100 | 15 | 100 | 100 | 16 | 50 | 100 | 14 | 100 | 100 |
| **18** | 18 | 50 | 50 | 15 | 25 | 50 | 12 | 50 | 50 | 13 | 50 | 50 | 14 | 25 | 50 |

**Table 13: Antimicrobial activities of the tested compounds (Cont.)**

| Comp. No. | ***S. aureus*** (DSM 1104) | | | ***B. subtilis*** (ATCC 6633) | | | ***P. aeruginosa*** (ATCC 10145) | | | ***E. coli*** (ATCC 11775) | | | ***C. albicans*** (DSM 70014) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *IZ* | *MIC* | *MBC* | *IZ* | *MIC* | *MBC* | *IZ* | *MIC* | *MBC* | *IZ* | *MIC* | *MBC* | *IZ* | *MIC* | *MFC* |
| **19** | 18 | 100 | 100 | 15 | 50 | 50 | 15 | 25 | 50 | 16 | 100 | 100 | 14 | 100 | 100 |
| **20a** | 14 | 100 | 200 | 16 | 100 | 100 | 14 | 100 | 100 | 18 | 50 | 100 | 12 | 100 | 100 |
| **20b** | 12 | 50 | 100 | 16 | 100 | 100 | 15 | 50 | 100 | 18 | 50 | 50 | 17 | 100 | 100 |
| **21** | 18 | 25 | 50 | 14 | 100 | 100 | 12 | 50 | 50 | 14 | 25 | 50 | 16 | 25 | 50 |
| **22a** | 15 | 100 | 100 | 18 | 50 | 100 | 14 | 100 | 100 | 12 | 50 | 50 | 16 | 50 | 100 |
| **22b** | 18 | 100 | 200 | 16 | 100 | 100 | 12 | 100 | 100 | 14 | 50 | 100 | 14 | 100 | 100 |
| **23a** | 18 | 50 | 50 | 14 | 25 | 50 | 12 | 25 | 50 | 14 | 50 | 50 | 16 | 50 | 50 |
| **23b** | 16 | 50 | 50 | 14 | 25 | 50 | 14 | 25 | 50 | 16 | 50 | 50 | 16 | 50 | 50 |
| **24a** | 14 | 100 | 100 | 18 | 50 | 50 | 16 | 25 | 50 | 14 | 100 | 100 | 14 | 100 | 100 |
| **24b** | 15 | 50 | 50 | 14 | 25 | 50 | 16 | 25 | 50 | 18 | 50 | 50 | 14 | 50 | 50 |
| **25** | 15 | 50 | 50 | 16 | 50 | 50 | 14 | 50 | 100 | 11 | 50 | 100 | 18 | 50 | 50 |
| **26a** | 14 | 25 | 50 | 18 | 50 | 50 | 16 | 50 | 50 | 14 | 25 | 50 | 12 | 50 | 50 |
| **26b** | 14 | 50 | 50 | 16 | 50 | 50 | 14 | 50 | 100 | 18 | 50 | 100 | 14 | 50 | 50 |
| **27a** | 14 | 25 | 25 | 16 | 50 | 50 | 16 | 50 | 50 | 17 | 25 | 50 | 12 | 50 | 50 |
| **27b** | 15 | 50 | 50 | 14 | 50 | 50 | 15 | 50 | 100 | 18 | 50 | 100 | 18 | 50 | 50 |
| **27c** | 15 | 25 | 50 | 12 | 50 | 50 | 14 | 50 | 100 | 16 | 50 | 100 | 18 | 50 | 100 |
| **Ampicillin** | | 5 | | | 12.5 | | | 50 | | | 10 | | | | |
| **Clotrimazole** | | | | | | | | | | | | | | 5 | |