# Synthesis and Antimicrobial Activities of Some New Tetrahydro-2H-1,3,5-thiadiazine-2-thione Derivatives of Ampicillin

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Compounds having α-[dihydro-5-substituted 6-thioxo- 2H-1,3,5-thiadiazine-3(4H)-yl]benzylpenicillin structure were synthesized by the reaction of ampicillin trihydrate, formaldehyde and dithiocarbamic acid salts. The structures were evident from chemical and spectral analysis. The antimicrobial activities of the compounds were investigated against some gram-positive (*Staphylococcus aureus* and *Streptococcus faecalis*) and gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria and some yeast-like fungi (*Candida albicans, C. parapsilosis, C. stellatoidea* and *C. pseudotropicalis*) and molds such as *Trichophyton rubrum, T. mentagrophytes, Microsporum canis, M. gypseum, Penicillium* and *Aspergillus* by the tube dilution method. In addition to MIC (minimal inhibitory concentration), MBC (minimal bactericidal concentration) and MFC (minimal fungicidal concentration) values were determined using ampicillin trihydrate as standard. The compounds synthesized were usually found as effective as ampicillin trihydrate against *S. aureus* and *S. faecalis* and less effective than ampicillin trihydrate against *E. coli*. Both the compounds synthesized and ampicillin trihydrate are ineffective in the concentrations studied against *P. aeruginosa*. Compound **10** and **11** are more effective against all the yeast-like fungi than the other compounds and ampicillin trihydrate.

**Synthese und antimikrobielle Aktivität einiger neuer Tetrahydro-2H-1,3,5-thiadiazin-2-thion-Derivate des Ampicillins**

Einige Derivate des α-[Dihydro-5-substituierten 6-thioxo- 2H-1,3,5-thiadiazin-3(4H)yl]benzylpenicillins wurden aus Ampicillintrihydrat, Formaldehyd und Dithiocarbaminsäure-Salzen hergestellt. Die Strukturen der Substanzen wurden durch chemische und spektrale Analyse gesichert. Die antimikrobiellen Aktivitäten dieser Derivate und des Ampicillintrihydrats (als Vergleich) wurden gegen einige gram-positive Bakterien (*Staphylococcus* und *Streptococcus faecalis*) und gram-negative Bakterien (*Escherichia coli* und *Pseudomonas aeruginosa*), einige Candidahefen (*C. albicans, parapsilosis, stellatoidea* und *pseudotropicalis*) und Fungi (*Trichophyton rubrum, T. mentagrophytes, Microsporum canis, M. gypseum, Penicillium* und *Aspergillus*) im Reihenverdünnungstest untersucht; die minimale Hemmkonzentration (MHK), minimale bakterizide Konzentration (MBK) und minimale fungizide Konzentration (MFK) wurden festgestellt. Alle synthetisierten Derivate und Ampicillintrihydrat sind gleich wirksam gegen *S. aureus* und *S. faecalis*, aber die Wirkung der synthetisierten Substanzen gegen *E. coli* ist schwächer als die des Ampicillintrihydrats. Sowohl die synthetisierten Derivate als auch Ampicillintrihydrat haben keine Aktivität gegen *P. aeruginosa* in der angegebenen Konzentration. **10** und **11** sind aktiver als die anderen Derivate und als Ampicillintrihydrat gegen Candidahefen.

Although ampicillin has a special importance among β-lactam antibiotics because of its considerable acid stability and its activity against both gram-positive and gram-negative bacteria[1,2], its activity against some bacteria such as *Klebsiella, Enterobacter, Serratia*, indol-positive *Proteus* species, *Bacteroides fragilis* and *P. aeruginosa* is not sufficient[2,3]. So, two fundamental points should be considered to synthesize new ampicillin derivatives:

a) To improve oral absorption for optimum pharmacokinetic features
and b) To increase effectiveness of ampicillin against some gram-negative bacteria for a broad antibacterial spectrum.

As a matter of fact, it was reported that improved oral absorption and a broad antibacterial spectrum were achieved by esterification of the carboxyl group (pivampicillin[4], bacampicillin[5], talampicillin[6], sultamicillin[7]) and the reaction of the α-amino group on the side chain with an aldehyde (hetacillin[8]), a ketone (metampicillin[9]) or various heterocyclic acid chlorides (azlocillin, mezlocillin, piperacillin[10-15], respectively.

On the other hand, it is well known that the tetrahydro-2H-1,3,5-thiadiazine-2-thione ring shows antifungal activity by hydrolyzing in aqueous media to antifungal isothiocyanates[16,17-19].

So in this study new ampicillin derivatives (except compound **1**[20]) with α-[dihydro-5-substituted 6-thioxo- 2H-1,3,5-thiadiazine-3(4H)yl]benzylpenicillin structure were synthesized as prodrugs which are thought to have a broader antibacterial spectrum than the parent compound also to be effective on secondary fungal infections, by making the N-atom of the α-amino group in ampicillin trihydrate a member of the tetrahydro-2H-1,3,5-thiadiazine-2-thione ring (Tab. 1).

## *Results and Discussion*

The compounds were synthesized from proper alkyl- or aralkylamines. In the first step of the reaction, dithiocarbamic acid salts were formed by reacting primary amines with $CS_2$ and KOH[21-23]. Then the tetrahydro-2H-1,3,5-thiadiazine-2-thione ring was formed by the reaction of the dithiocarbamic acid salts with formaldehyde and ampicillin trihydrate:

Instead of using a two fold excess of formaldehyde as reported[21-23], we used a 10% excess of formaldehyde to increase the reaction yield. On the other hand, we observed that the amount of ampicillin trihydrate should be less than the amount of dithiocarbamic acid salts, because when the dithiocarbamic acid salts were used in equimolar amounts to ampicillin trihydrate unreacted ampicillin trihydrate

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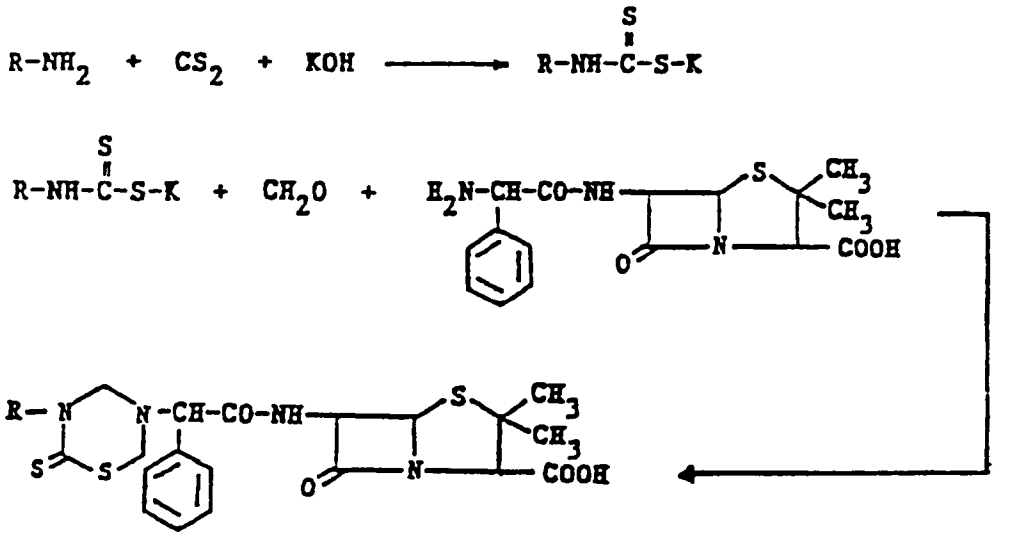
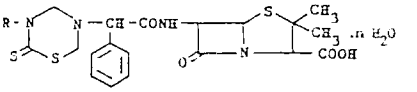


could not be removed from the reaction medium. Ampicillin trihydrate was added to the reaction medium in pH 7.8 phosphate buffer, because ampicillin trihydrate is readily hydrolyzed at acidic[24-25] and basic pH[24] and the tetrahydro-2H-1,3,5-thiadiazine-2-thione ring is not stable in aqueous solution (< pH 6.8)[19]. Furthermore, the acidic isolation of the products from the reaction medium was performed below 0°C to prevent hydrolysis of ampicillin trihydrate[26].

In the UV-spectra of all the compounds, two strong absorption bands were seen (250 and 290 nm) which were attributed to the tetrahydro-2H-1,3,5-thiadiazine-2-thione ring[23,27-29].- In the IR spectra the N-H-stretching bands of the benzylic ampicillin trihydrate did not appear on account of the cyclization.- In the $^1$H-NMR spectra, alkyl protons of the N-3- side chains (except N-$CH_3$) of the tetrahydro-2H-1,3,5-thiadiazine-2-thione ring and the $CH_3$-protons at C-2 were seen together as a multiplet at 0.40-2.25 ppm. The N-$CH_3$-protons at N-3 of the tetrahydro-2H-1,3,5-thiadiazine-2-thione ring resonate at 3.00-4.00 ppm[30]. The signals of H-4 and H-6 of the tetrahydro-2H-1,3,5-thiadiazine-2-thione ring, H-3 of ampicillin trihydrate, the methine proton of the side chain and the β-lactam ring protons were observed as a multiplet together with the signals of HOD at 4.00-6.00 ppm[26,30,31]. The amid proton appeared at ≈ 9.00 ppm as a doublet with J = 9.1 Hz (Tab. 2).- In the positive ion and negative ion FAB mass spectra [M+H] and [M-H] ions were seen[32,33].

Antibacterial and antifungal activities of the compounds synthesized and of ampicillin trihydrate were investigated. The compounds were usually found as effective as ampicillin trihydrate against gram-positive bacteria and less effective than ampicillin trihydrate against E. coli. Furthermore all the compounds tested were found ineffective against *P. aeruginosa* (MIC 100 µg/ml.) (Tab. 3). On the other hand, the compounds having benzyl[**10**] and phenethyl[**11**] substituents at N-3 of the tetrahydro-2H-1,3,5-thiadiazine-2-thione ring are more potent against yeast-like fungi than the other compounds (Tab. 4). These results are in accordance with the lit., because it was reported that phenethyl and benzyl isothiocyanates which are formed by hydrolysis of

**Tab. 1: Melting points, yield, empiric formulae, and elementary analyses of compounds 1-11.**



| Comp. | R | n | M.p. °C | Yield % | Empiric Formula | Elementary Analysis Calc.% Found% |
|---|---|---|---|---|---|---|
| 1 | $CH_3$- | 1/2 | 170-74 | 53 | $C_{20}H_{24}N_4O_4S_3 \cdot$ 1/2 $H_2O$ | C:49.05 C:49.30 H: 5.15 H: 5.50 N:11.44 N:11.10 |
| 2 | $C_2H_5$- | 1 1/2 | 150-54 | 66 | $C_{21}H_{26}N_4O_4S_3 \cdot$ 1 1/2 $H_2O$ | C:48.35 C:48.10 H: 5.60 H: 5.60 N:10.74 N:10.70 |
| 3 | n-$C_3H_7$- | 1 1/2 | 157-60 | 62 | $C_{22}H_{28}N_4O_4S_3 \cdot$ 1 1/2 $H_2O$ | C:49.33 C:49.60 H: 5.83 H: 5.80 N:10.46 N:10.80 |
| 4 | n-$C_4H_9$- | 1 1/2 | 148-52 | 66 | $C_{23}H_{30}N_4O_4S_3 \cdot$ 1 1/2 $H_2O$ | C:50.25 C:50.40 H: 6.06 H: 6.00 N:10.19 N:10.50 |
| 5 | n-$C_5H_{11}$- | 1 | 143-47 | 68 | $C_{24}H_{32}N_4O_4S_3 \cdot$ 1 $H_2O$ | C:51.96 C:51.60 H: 6.18 H: 6.10 N:10.10 N:10.40 |
| 6 | i-$C_3H_7$- | 1 | 166-69 | 66 | $C_{22}H_{28}N_4O_4S_3 \cdot$ 1 $H_2O$ | C:50.17 C:49.90 H: 5.74 H: 5.70 N:10.64 N:10.60 |
| 7 | i-$C_4H_9$- | 2 | 150-53 | 83 | $C_{23}H_{30}N_4O_4S_3 \cdot$ 2 $H_2O$ | C:49.44 C:49.40 H: 6.13 H: 6.10 N:10.03 N:10.40 |
| 8 | i-$C_5H_{11}$- | 2 | 144-47 | 83 | $C_{24}H_{32}N_4O_4S_3 \cdot$ 2 $H_2O$ | C:50.33 C:50.40 H: 6.33 H: 6.30 N: 9.78 N:10.10 |
| 9 |  | 1 1/2 | 168-72 | 70 | $C_{24}H_{26}N_4O_5S_3 \cdot$ 1 1/2 $H_2O$ | C:50.24 C:50.50 H: 5.09 H: 5.40 N: 9.77 N:10.10 |
| 10 |  | 1 1/2 | 149-52 | 80 | $C_{26}H_{28}N_4O_4S_3 \cdot$ 1 1/2 $H_2O$ | C:53.50 C:53.30 H: 5.35 H: 5.40 N: 9.60 N:10.10 |
| 11 |  | 1 1/2 | 141-44 | 71 | $C_{27}H_{30}N_4O_4S_3 \cdot$ 1 1/2 $H_2O$ | C:54.25 C:54.10 H: 5.56 H: 5.50 N: 9.37 N: 9.70 |

Tab. 2: Spectral data of compounds **1-11**.

| Comp. | $UV_{\lambda max.}^{MeOH}$ (log ε) | IR (KBr) ($cm^{-1}$) | $^1$H-NMR (DMSO-$d_6$) (ppm) |
|---|---|---|---|
| 1 | 285 (3.52)<br>323 (3.07) | 3590-3140, 3070, 3040, 2980, 2940, 1780, 1695, 1510, 755, 700 | 0.75-1.87 (6H; m), 3.12 (3H; s), 4.00-6.00 (8H; m) 7.00-7.75 (5H; m), 9.27 (1H; d) |
| 2 | 250 (3.71)sh[x]<br>287 (3.89)<br>326 (3.03)sh | 3560-3140, 3070, 3040, 2980, 2940, 1780, 1740, 1685, 1500, 750, 700 | 0.62-1.75 (9H; m), 3.12-4.00 (2H; m), 4.20-6.00 (8H; m), 7.00-7.75 (5H; m) 9.32 (1H; d). |
| 3 | 253 (3.65)sh<br>288 (3.68)<br>335 (2.72)sh | 3560-3130, 3040, 2970, 2940, 1780, 1685, 1505, 745, 700 | 0.40-2.00 (11H; m), 3.12-3.87 (2H; m), 3.94-6.25 (8H; m), 7.00-7.75 (5H; m) 9.33 (1H; d) |
| 4 | 248 (3.79)sh<br>287 (3.93)<br>330 (3.10)sh | 3580-3140, 3040, 2970, 2940, 1785, 1745, 1685, 1500, 755, 700 | 0.50-1.75 (13H; m), 3.18-4.00 (2H; m), 4.20-6.25 (8H; m), 7.12-7.75 (5H; m) 9,35 (1H; d). |
| 5 | 248 (3.97)sh<br>287 (3.88)<br>332 (2.83)sh | 3580-3140, 3070, 3040, 2970, 2940, 1785, 1730, 1695, 1505, 750, 700 | 0.56-1.80 (15H; m), 3.31-4.00 (2H; m), 4.06-6.37 (8H; m), 7.12-7.75 (5H; m) 9.34 (1H; d) |
| 6 | 250 (3.53)sh<br>278 (3.31)<br>328 (2.92) | 3580-3140, 3080, 3040, 2970, 2930, 1780, 1730, 1680, 1500, 750, 700 | 0.65-1.87 (12H; m), 2.87-3.87 (1H; m), 3.94-6.50 (8H; m), 6.87-7.87 (5H; m) 10.06 (1H; d) |
| 7 | 248 (3.77)<br>289 (3.85)<br>328 (2.94)sh | 3560-3130, 3040, 2970, 2930, 1780, 1730, 1680, 1500, 750, 700 | 0.40-2.25 (13H; m), 3.05-3.81 (2H; m), 3,94-5.81 (8H; m), 7.03-7.68 (5H; m) 9.31 (1H; d) |
| 8 | 248 (3.80)sh<br>287 (3.87)<br>332 (2.96)sh | 3560-3140, 3070, 3040, 2970, 2940, 1785, 1740, 1685, 1500, 755, 700 | 0.41-1.93 (15H; m), 3.06-3.94 (2H; m), 4.06-6.56 (8H; m), 7.00-7.87 (5H; m) 9.36 (1H; d) |
| 9 | 289 (3.32)<br>322 (2.87)sh | 3560-3140, 3100, 3040, 2980, 2940, 1780, 1720, 1690, 1500, 750, 700 | 0.93-2.06 (6H; m), 3.18-3.87 (2H; m), 3.94-6.25 (10H; m), 7.00-7.87 (6H; m); 8.75 (1H; d) |
| 10 | 250 (3.87)<br>290 (3.89)<br>332 (2.94)sh | 3560-3140, 3070, 3040, 2980, 2930, 1780, 1740, 1690, 1500, 750, 700 | 1.31 (3H; s), 1.43 (3H; s) 3.18-3.87 (2H; m), 4.00-6.20 (8H; m), 7.06-7.68 (10H; m), 9.30 (1H; d) |
| 11 | 248 (3.78)<br>287 (3.79)<br>327 (2.41)sh | 3560-3140, 3070, 3040, 2980, 2940, 1785, 1730, 1700, 1500, 750, 700 | 0.56-1.87 (6H; m), 2.62-3.12 (2H; m), 3.25-6.20 (10H; m), 6.75-7,75 (10H; m), 9.39 (1H; d) |

[x] sh: shoulder

[x]sh: shoulder

the tetrahydro-2H-1,3,5-thiadiazine-2-thione ring in the aqueous media are more effective than other alkyl isothiocyanates against fungi[34,35]. Although these compounds are preparated as pro-drugs, their *in-vitro* antimicrobial activity results are hopeful except the activity against molds (Tab. 5). Because of high activities of compounds **10** and **11** against yeast-like fungi we think that they could bring an advantage for secondary candida infectious which occur under penicillin treatment.

## Experimental Part

### *Chemistry*

All chemicals used except ampicillin trihydrate (FAKO, Pharmaceutical Firm, ISTANBUL) were supplied from Merck.- m.p.s.: Thomas-Hoover capillary melting point apparatus, uncorrected.- UV spectra: Hitachi 220S.- IR spectra: Perkin Elmer 298 (KBr pellets).- $^1$H-NMR spectra: Bruker AC 80 MHz, TMS as int. standard, chemical shifts in δ values (ppm).- Mass spectra: EI- and FAB-MS: (Institute of Pharmacy, Regensburg University, West Germany).- Elementary analysis (C,H,N): Knoll-BASF, Ludwigshafen, West Germany.

### *α-[Dihydro-5-substituted 6-thioxo-2H-1,3,5-thiadiazine-3(4H)yl] benzylpenicillins*

$CS_2$ (0.6 ml, 0.06 mole) was added to a stirred mixture of primary amine (0.01 mole) and KOH (20%, 2.8 ml, 0.01 mole). The mixture was stirred for 3 h at room temp. Then formaldehyde solution (37%, 1.63 ml, 0.022 mole) was added to the mixture and stirring was continued for 30 min; an oily residue was formed which was removed by filtration. The clear filtrate obtained was added dropwise to a stirred ampicillin trihydrate suspension (3.42 g, 0.0085 mole) in pH 7.8 phosphate buffer and the mixture was stirred for 4 h and kept in a refrigerator overnight. Then the mixture was extracted three times with ether (15 ml). The org. layer was removed. The aqueous solution was cooled in an ice-bath and acidified by dilute HCl (pH 2). The mixture was stirred for 30 min at 0°C and the precipitate formed was filtered, washed with cold water and dried in a refrigerator.

### *Microbiology*

*Mueller-Hinton Broth* (Oxoid), Blood Agar (Difco) for bacteria and *Sabouraud* Dextrose Broth (Difco) and *Sabouraud* Dextrose Agar (Difco) for

Tab. 3: Antibacterial activity of compounds **1-11** (MIC and MBC in μg/ml).

| Comp. | *Staph. aureus* (ATCC 6538) | | *Strep. faecalis* (ATCC 10541) | | *E. coli* (ATCC 25922) | | *P. aeruginosa* (ATCC 27853) | |
|---|---|---|---|---|---|---|---|---|
| | MIC | MBC | MIC | MBC | MIC | MBC | MIC | MBC |
| **1** | 3.12 | 12.5 | 3.12 | 3.12 | 12.5 | 12.5 | 100 | - |
| **2** | 3.12 | 6.25 | 3.12 | 12.5 | 12.5 | 25 | 100 | - |
| **3** | 1.56 | 12.5 | 3.12 | 12.5 | 12.5 | 25 | 100 | - |
| **4** | 3.12 | 12.5 | 6.25 | 25 | 25 | 25 | 100 | - |
| **5** | 3.12 | 6.25 | 6.25 | 12.5 | 25 | 37.5 | 100 | - |
| **6** | 12.5 | 50 | 3.12 | 25 | 25 | 25 | 100 | - |
| **7** | 25 | 75 | 12.5 | 12.5 | 25 | 25 | 100 | - |
| **8** | 12.5 | 25 | 12.5 | 25 | 25 | 25 | 100 | - |
| **9** | 6.25 | 25 | 3.12 | 25 | 12.5 | 25 | 100 | - |
| **10** | 6.25 | 25 | 25 | 25 | 25 | 37.5 | 100 | - |
| **11** | 12.5 | 25 | 12.5 | 12.5 | 25 | 25 | 100 | - |
| Amp.[x] | 3.12 | 6.25 | 3.12 | 3.12 | 6.25 | 6.25 | 100 | - |

[x] Amp.: Ampicillin trihydrate

Tab. 4: Antifungal activity of compounds **1-11** against yeast-like fungi (MIC and MFC in μg/ml)

| Comp. | *C. albicans* | | *C. parapsilosis* | | *C. stellatoidea* | | *C. pseudotropicalis* | |
|---|---|---|---|---|---|---|---|---|
| | MIC | MFC | MIC | MFC | MIC | MFC | MIC | MFC |
| **1** | 100 | - | 100 | - | 100 | - | 100 | - |
| **2** | 100 | - | 100 | - | 100 | 100 | 100 | - |
| **3** | 100 | - | 100 | 100 | 50 | | 100 | - |
| **4** | 100 | - | 100 | - | 100 | - | 100 | - |
| **5** | 100 | - | 100 | - | 100 | 100 | 100 | - |
| **6** | 100 | - | 100 | - | 37.5 | 50 | 100 | - |
| **7** | 100 | - | 100 | 100 | 100 | 100 | 100 | - |
| **8** | 100 | - | 100 | - | 25 | 37.5 | 100 | - |
| **9** | 100 | - | 100 | - | 100 | 100 | 100 | - |
| **10** | 12.5 | 25 | 25 | 37.5 | 6.25 | 6.25 | 12.5 | 25 |
| **11** | 25 | 25 | 25 | 37.5 | 37.5 | 37.5 | 25 | 25 |
| Amp.[x] | 100 | - | 100 | - | 100 | - | 100 | - |

[x] Amp.: Ampicillin trihydrate

Tab. 5: Antifungal activity of compounds **1-11** against molds (MIC μg/ml).

| Comp. | *T. rubrum* | *T. mentagrophytes* | *M. canis* | *M. gypseum* | *Penicillium* | *Aspergillus* |
|---|---|---|---|---|---|---|
| **1** | 100 | 100 | 100 | 100 | 100 | 100 |
| **2** | 100 | 100 | 100 | 100 | 100 | 100 |
| **3** | 100 | 100 | 100 | 100 | 100 | 100 |
| **4** | 100 | 100 | 100 | 100 | 100 | 100 |
| **5** | 100 | 100 | 100 | 100 | 100 | 100 |
| **6** | 100 | 100 | 100 | 100 | 100 | 100 |
| **7** | 100 | 100 | 100 | 100 | 100 | 100 |
| **8** | 100 | 100 | 100 | 100 | 100 | 100 |
| **9** | 100 | 100 | 100 | 100 | 100 | 100 |
| **10** | 100 | 100 | 75 | 100 | 100 | 100 |
| **11** | 100 | 100 | 75 | 75 | 100 | 100 |
| Amp.[x] | 100 | 100 | 100 | 100 | 100 | 100 |

[x] Amp.: Ampicillin trihydrate

fungi were used as media. The tube dilution method was used for the determination of MIC values[37]. The concentrations of the compounds in tubes were 100; 75; 50; 37.5; 25; 12.5; 6.25; 3.12; 1.56; 0.78, and 0.39 μg/ml, respectively, and the final inoculum size was $10^5$-$10^6$ c.f.u./ml. MBC and MFC values of the compounds were determined by subculturing a known quantity of inoculum from each tube of broth that showed no visible turbidity after incubation period to solid agar plates. Ampicillin trihydrate was used as standard in the microbiological studies.

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