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Synthesis, characterization and biological activity of novel 3-benzyl-2-phenyl-4(5*H*)-(substituted phenylhydrazino)-1,3-oxazolidines derivatives

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ABSTRACT

*A series of some new 3-benzyl-2-phenyl-4(5H)-(substituted phenylhydrazino)-1, 3-oxazolidines **6a-f** derivatives were synthesized. The in vitro anti-bacterial and anti-fungal activities were determined by paper disk diffusion method. The minimum inhibitory concentrations (MIC) of the compounds were also determined by agar streak dilution method. Most of the synthesized compounds exhibited significant anti-bacterial and anti-fungal activities. Among the synthesized compounds 3-benzyl-2-phenyl-4(5H)-4'methoxyphenyl hydrazino-1, 3-oxazolidine (6d) and 3-benzyl-2-phenyl-4(5H)-4'acetamidophenyl hydrazino-1, 3-oxazolidine (**6e**) exhibited most potent in vitro antimicrobial activity against Staphylococcus aureus, Staphylococcus epidermidis, Micrococcus luteus, Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Aspergillus niger and Candida albicans.*

Key words: Oxazolidine; Substituted phenylhydrazine; Anti-bacterial; Anti-fungal.

INTRODUCTION

The oxazolidine compounds class is particularly well recognized for its activity against clinically important susceptible and resistant Gram-positive bacteria such as methicillin-resistant Staphylococcus aureus, vancomycin-resistant Enterococcus faecium, and penicillin-resistant Streptococcus pneumouniae [1]. A new class (1*H*-1,2,3)-triazole C-5 substituted oxazolidin-2-

ones was described [2]. These oxazolidine showed impressive antibacterial activity and were less prone to inhibit MAOs. The oxazolidine moiety is present in a number of compounds which show diverse biological activities such as mono amine oxidase (MAO) inhibition [3], GP IIb/IIIa antagonism [4] neuroleptic activity [5] and antibacterial activity [6]. As documented in the literature, many oxazolidine act as Mycobacterium sp activity [7]. Due to this medicinal importance, methods for generating libraries of these compounds have received significant interest [8]. These observation led to the conception that a novel series of 3-benzyl-2-phenyl-4(5*H*)-(substituted phenylhydrazino)-1, 3-oxazolidines **6a-f** derivatives were synthesized and their chemical structure were confirmed by IR, ^{1}H-NMR, ^{13}C-NMR, mass spectral and elemental analyses. These compounds were screened for their antimicrobial activities.

MATERIALS AND METHODS

Materials

Synthetic starting material, reagents and solvents were of analytical reagent grade or of the highest quality commercially available and were purchased from Aldrich Chemical Co., Merck Chemical Co. and were dried when necessary.

The melting points were taken in open capillary tube and are uncorrected. IR spectra were recorded with KBr pellets (ABB Bomem FT-IR spectrometer MB 104 ABB Limited Bangaluru, India). ^{1}H-NMR, ^{13}C-NMR spectra (Bruker 400 NMR spectrometer Mumbai, India) were recorded with TMS as internal references. Mass spectral data were recorded with a quadrupol mass spectrometer (Shimadzu GC MS QP 5000, Chennai, India), and microanalyses were performed using a *vario EL* V300 *elemental* analyzer (*Elemental* Analysensysteme *GmbH* Chennai, India). The purity of the compounds was checked by TLC on pre-coated SiO_2 gel (HF_{254}, 200 mesh) aluminium plates (E.Merck) using ethyl acetate: benzene (1:3) and visualized in UV chamber. IR, ^{1}H-NMR, mass spectral datas and elemental analyses were consistent with the assigned structures.

***General Procedures*.** The target novel oxazolidine derivatives were synthesized by previously reported method [9]. Accordingly, benzylamine **1** was treated with an equimolar amount of benzaldehyde **2** and an hydroxy acetic acid **3** in dry toluene under reflux 24- 48 h to give 3-benzyl-2-phenyl-1,3-oxazolidine-4(5*H*)-one **4**, further its treat with thionyl chloride and DMF to get chloro derivative **5** 3-benzyl-2-phenyl-4(5H)-chloro-1,3-oxazolidine and then coupled with substituted phenyl hydrazine, anhydrous sodium acetate and glacial acetic acid were dissolved in warm ethanol and refluxed for 30 min. After standing for approximately 24 h at room temperature, the product were separated by filtration, vaccum dried and recrystallized from warm ethanol to yields 3-benzyl-2-phenyl-4(5*H*)-(substituted phenyl hydrazino)-1,3-oxazolidines **6a-f**.

3-benzyl-2-phenyl-1, 3-oxazolidine-4(5H)-one (4)

Yellow Solid; Yield: 82%; mp. 172-174°C, IR : 3096 (Ar-CH), 1728 (C=O), 1468 (C=C) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 6.96-7.54 (m,10H, Ar-H), 6.67 (s, 1H, -CH), 4.12-4.62 (m, 4H, 2 × CH_2); ^{13}C-NMR ($CDCl_3$): δ 43.01, 67.12, 80.60, 127.08, 127.12, 128.07, 128.09, 128.21, 128.27, 128.49, 128.76, 128.87, 128.89, 135.42, 136.53, 169.68; EI-MS (m/z, %): 253 [M]+; (Calcd for $C_{16}H_{15}NO_2$; 253.3). Anal. Calcd for $C_{16}H_{15}NO_2$, C, 75.87; H, 5.97; N, 5.53; Found: C, 75.46; H, 5.61; N, 5.36.

3-benzyl-2-phenyl-4(5H) chloro-1, 3-oxazolidine (5)
Pale Yellow Solid; Yield: 71%; mp. 146-148°C, IR : 3084 (Ar-CH), 827 (C-Cl), 1412 (C=C) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 6.18-7.42 (m, 10H, Ar-H), 4.98-5.27 (s, 2H, -CH), 3.46-4.22 (m, 4H, 2 × CH_2); ^{13}C-NMR ($CDCl_3$): δ 48.70, 79.24, 80.86, 93.48, 127.06, 127.16, 128.21, 128.37, 128.42, 128.71, 128.88, 128.92, 128.97, 128.99, 135.14, 136.42; EI-MS (m/z, %): 273 [M]+; (Calcd for $C_{16}H_{16}ClNO$; 273.09). Anal. Calcd for $C_{16}H_{16}ClNO$, C, 70.20; H, 5.89; N, 5.12; Found: C, 70.41; H, 5.96; N, 5.72.

3-benzyl-2-phenyl-4(5H)-4'bromophenyl hydrazino-1, 3-oxazolidine (6a)
Yellow Crystals; Yield: 74%; mp. 171-173°C, IR : 3031 (Ar-CH), 813 (C-Br), 1496 (C=C), 1309 (N-H bending), 3389 (N-H stretching) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 6.89-7.49 (m, 14H, Ar-H), 5.19 (s, 2H, -CH), 7.49 (s, 2H, N-H), 3.56-3.69 (m, 4H, 2 × CH_2); ^{13}C-NMR ($CDCl_3$): δ 48.64, 75.62, 82.06, 93.48, 113.54, 115.12, 115.42, 127.14, 127.32, 128.28, 128.34, 128.61, 128.62, 128.66, 128.74, 128.83, 128.92, 132.61, 132.64, 134.42, 135.66, 150.06; EI-MS (m/z, %): [M+2] 425; (Calcd for $C_{22}H_{22}BrN_3O$; 425.33). Anal. Calcd for $C_{22}H_{22}BrN_3O$; C, 62.27; H, 5.23; N, 9.90; Found: C, 62.11; H, 5.22; N, 9.74.

3-benzyl-2-phenyl-4(5H)-4'chlorophenyl hydrazino-1, 3-oxazolidine (6b)
Pale Yellow Solid; Yield: 78%; mp. 175-177°C, IR : 3029 (Ar-CH), 829 (C-Cl), 1484 (C=C), 1316 (N-H bending), 3391 (N-H stretching) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 6.87-7.42 (m, 14H, Ar-H), 5.22 (s, 2H, -CH), 7.51 (s, 2H, N-H), 3.49-3.57 (m, 4H, 2 × CH_2); ^{13}C-NMR ($CDCl_3$): δ 48.62, 75.67, 82.01, 93.48, 113.57, 115.19, 115.41, 127.18, 127.32, 128.28, 128.31, 128.63, 128.67, 128.68, 128.71, 128.83, 128.94, 132.62, 132.61, 134.44, 135.67, 150.12; EI-MS (m/z, %): [M+2] 381; (Calcd for $C_{22}H_{22}ClN_3O$; 381.15). Anal. Calcd for $C_{22}H_{22}ClN_3O$; C, 69.56; H, 5.84; N, 11.06; Found: C, 69.51; H, 5.96; N, 11.12.

3-benzyl-2-phenyl-4(5H)-4'fluorophenyl hydrazino-1, 3-oxazolidine (6c)
Pale Brown Solid; Yield: 71%; mp. 170-172°C, IR : 3021 (Ar-CH), 810 (C-F), 1481 (C=C), 1326 (N-H bending), 3397 (N-H stretching) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 6.81-7.43 (m, 14H, Ar-H), 5.25 (s, 2H, -CH), 7.54 (s, 2H, N-H), 3.42-3.52 (m, 4H, 2 × CH_2); ^{13}C-NMR ($CDCl_3$): δ 48.61, 75.67, 82.12, 93.41, 113.53, 115.15, 115.41, 127.12, 127.31, 128.24, 128.32, 128.63, 128.61, 128.63, 128.71, 128.82, 128.91, 132.67, 132.69, 134.41, 135.61, 150.01; EI-MS (m/z, %): [M] 381; (Calcd for $C_{22}H_{22}FN_3O$; 363.17). Anal. Calcd for $C_{22}H_{22}FN_3O$; C, 72.71; H, 6.10; N, 11.56; Found: C, 72.62; H, 6.19; N, 11.42.

3-benzyl-2-phenyl-4(5H)-4'methoxyphenyl hydrazino-1, 3-oxazolidine (6d)
Pale Solid; Yield: 71%; mp. 181-183°C, IR : 3012 (Ar-CH), 1426 (C=C), 1331 (N-H bending), 3326 (N-H stretching) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 6.83-7.47 (m, 14H, Ar-H), 5.27 (s, 2H, -CH), 7.48 (s, 2H, N-H), 3.16 (s, 3H, -OCH_3) 3.36-4.85 (m, 4H, 2 × CH_2); ^{13}C-NMR ($CDCl_3$): δ 47.67, 55.42, 75.72, 82.62, 93.71, 113.57, 115.19, 115.49, 127.17, 127.38, 128.26, 128.33, 128.67, 128.65, 128.64, 128.74, 128.87, 128.98, 132.66, 132.72, 134.42, 135.66, 150.16; EI-MS (m/z, %): [M] 375; (Calcd for $C_{23}H_{25}N_3O_2$; 375.19). Anal. Calcd for $C_{23}H_{25}N_3O_2$; C, 73.57; H, 6.71; N, 11.19; Found: C, 73.43; H, 6.76; N, 11.21.

3-benzyl-2-phenyl-4(5H)-4'acetamidophenyl hydrazino-1, 3-oxazolidine (6e)
Pale Brown Solid; Yield: 76%; mp. 176-178°C, IR : 3024 (Ar-CH), 1423 (C=C), 1731 (C=O), 1337 (N-H bending), 3316 (N-H stretching) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 6.76-7.32 (m, 14H, Ar-H), 5.29 (s, 2H, -CH), 7.29 (s, 3H, N-H), 3.19 (s, 3H, $-CH_3$) 3.31-4.82 (m, 4H, 2 × CH_2); ^{13}C-NMR ($CDCl_3$): δ 22.92, 48.61, 68.19, 75.63, 82.16, 93.18, 113.14, 115.12, 115.17, 127.14, 127.23, 128.12, 128.26, 128.34, 128.53, 128.62, 128.74, 128.76, 128.78, 132.61, 132.64, 134.12, 135.66, 150.11; EI-MS (m/z, %): [M] 402; (Calcd for $C_{24}H_{26}N_4O_2$; 402.21). Anal. Calcd for $C_{24}H_{26}N_4O_2$; C, 71.62; H, 6.51; N, 13.92; Found: C, 71.52; H, 6.59; N, 13.82.

3-benzyl-2-phenyl-4(5H)-benzyloxyphenyl hydrazino-1, 3-oxazolidine (6f)
Pale White Solid; Yield: 82%; mp. 182-184°C, IR : 3036 (Ar-CH), 1534 (C=C), 1326 (N-H bending), 3329 (N-H stretching) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 6.72-7.32 (m, 20H, Ar-H), 6.24 (s, 2H, -CH), 7.31 (s, 1H, N-H), 3.44-3.84 (m, 6H, 3 × CH_2); ^{13}C-NMR ($CDCl_3$): δ 48.44, 71.83, 75.62, 82.16, 93.48, 113.54, 115.42, 115.12, 127.62, 127.43, 127.26, 127.64, 127.32, 128.62, 128.46, 128.24, 128.13, 128.22, 128.34, 128.61, 128.28, 129.42, 129.17, 132.61, 132.64, 134.42, 135.66, 141.23, 150.02; EI-MS (m/z, %): [M]+ 451; (Calcd for $C_{29}H_{29}N_3O_2$; 451.23). Anal. Calcd for $C_{29}H_{29}N_3O_2$; C, 77.13; H, 6.74; N, 9.31; Found: C, 77.28; H, 6.62; N, 9.37.

Antimicrobial Screening
All the synthesized compounds were screened for anti-bacterial and anti-fungal activities by paper disc diffusion technique. The anti-bacterial activity of the compounds were evaluated against four gram positive bacteria (*Staphylococcus aureus* ATCC 9144, *Staphylococcus epidermidis* ATCC 155, *Micrococcus luteus* ATCC 4698 and *Bacillus cereus* ATCC 11778) and three gram negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 2853, *and Klebsiella pneumoniae* ATCC 11298*)*. The anti-fungal activities of the synthesized compounds were evaluated against two fungi (*Aspergillus niger* ATCC 9029 and *Candida albicans* ATCC 2091). The observed data on the antimicrobial activity of the synthesized compounds and standard drugs are given in Table 1.

Paper Disc Diffusion Technique
The sterilized (19) (autoclaved at 120°C for 30 minutes) medium (40-50°C) was inoculated (1 mL/100 mL of medium) with the suspension (10^5 cfu mL^{-1}) of the microorganism (matched to McFarland barium sulphate standard) and poured into a petridish to give a depth of 3-4 mm. The paper impregnated with the test compounds (µg mL^{-1} in dimethylformamide) was placed on the solidified medium. The plates were pre-incubated for 1 h at room temperature and incubated at 37°C for 24 and 48 h for anti-bacterial and anti-fungal activities, respectively. Ciprofloxacin (Dr. Reddy's Laboratories, Batch No: IC666E04, India) and Ketoconazole (Wuhan Shengmao Corporation, Batch No: SBML/403, China) were used as standard for anti-bacterial and anti-fungal activities, respectively. The observed zone of inhibition is presented in **Table 1**.

Minimum Inhibitory Concentration (MIC)
MIC (20) of the compound was determined by agar streak dilution method. A stock solution of the synthesized compound (100 µg mL^{-1}) in dimethylformamide was prepared and graded quantities of the test compounds were incorporated in specified quantity of molten sterile agar (nutrient agar for anti-bacterial activity and sabouraud dextrose agar medium for antifungal activity). A specified quantity of the medium (40-50°C) containing the compound was poured

into a petridish to give a depth of 3-4 mm and allowed to solidify. Suspension of the microorganism were prepared to contain approximately 10^5 cfu mL^{-1} and applied to plates with serially diluted compounds in dimethylformamide to be tested and incubated at 37°C for 24 h and 48 h for bacteria and fungi, respectively. The MIC was considered to be the lowest concentration of the test substance exhibiting no visible growth of bacteria or fungi on the plate. The observed MIC is presented in **Table 1**.

Statistical Analysis

Student's *t*-test was used to determine a significant difference between the control.

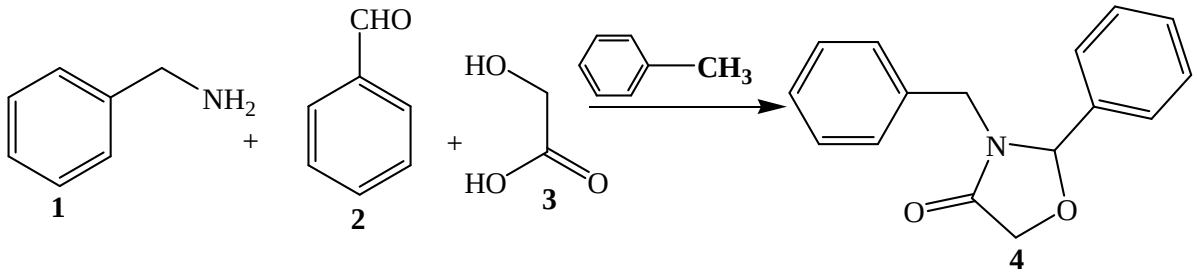
RESULTS AND DISCUSSION

Chemistry

The synthesized series of heterocycles, **6a-f** by the reaction of **5** with appropriate substituted phenyl hydrazine in the presence of anhydrous sodium acetate and glacial acetic acid as presented in **Scheme 1**. The IR, ^{1}H-NMR, ^{13}C-NMR , mass spectroscopy and elemental analysis for the new compound is in accordance with the assigned structures. The IR spectra of compounds **4** showed stretching bands of keto group at 1728 cm^{-1}. In **5**, stretching bands of chloro group at 749 cm^{-1} is evidence to conversion of oxazolidinone. The title compounds **6a-f** stretching and bending NH bands appear at 3300-3400 cm^{-1}, 1300-1350 cm^{-1} respectively. The recorded IR spectrum of representative compounds **6a-f** showed missing of chloro group bands. This clearly envisages that the chloro group of **5** is converted into secondary NH. The proton magnetic resonance spectra of oxazolidine and their corresponding derivatives have been recorded in $CDCl_3$. In this **6a-f** NH signal of 3-benzyl-2-phenyl-4(5*H*)-(4"-substituted phenylhydrazino)-1, 3-oxazolidines moiety appear at 7.49 (s), 7.51 (s), 7.54 (s), 7.48 (s), 7.29 (s), 7.31 (s) ppm respectively. The position and presence of NH signal in the ^{1}H-NMR spectra of final compounds conforms the secondary NH proton in oxazolidine moiety. This clearly envisages that oxazolidine-4(5*H*)-one moiety involve in 4(5*H*)-chloro-1,3-oxazolidine and further (4"- substituted phenyl hydrazino)-1,3-oxazolidines formation. All these observed facts clearly demonstrate that the 4th position of keto group in oxazolidine ring is converted into secondary amino group as indicated in **scheme 1** and conforms the proposed structure (**6a -f**)

Antimicrobial Screening

All the synthesized compounds exhibited moderate to good anti-bacterial and anti-fungal activity. Among the synthesized compounds, compounds **6e**, **6d** were found to possess significant anti-bacterial and anti-fungal activity when compared to standard drug Ciprofloxacin and Ketoconazole for anti-bacterial and anti-fungal activity respectively. Compound **6f** displayed moderate anti-microbial activity where as the remaining compounds shown good activity. The MIC of the synthesized compounds 3-benzyl-2-phenyl-4(5H)-(substituted phenylhydrazino) -1, 3-oxazolidines **6a-f** was established screened by agar streak dilution method with an MIC range of 8.1-26.7 µg mL^{-1}. The compound 3-benzyl-2-phenyl-4(5H)-4'acetamidophenylhydrazino -1, 3-oxazolidine (**6e**) was found to exhibit the highest anti-bacterial activity against *S.aureus* (8.1 µg mL^{-1}), *S.epidermidis* (10.7 µg mL^{-1}), *M.luteus* (10.1 µg mL^{-1}), *B.cereus* (10.6 µg mL^{-1}), *E.coli* (11.7 µg mL^{-1}), *P.aeruginosa* (11.1 µg mL^{-1}), *K.pneumoniae* (12.1 µg mL^{-1}), and **3**-benzyl-2-phenyl-4(5H)-4'methoxyphenyl hydrazino-1, 3-oxazolidine **(6d)** exhibited highest anti-fungal activity against *A.niger* (MIC: 9.4 µg mL^{-1}) and *C.albicans* (MIC: 10.5 µg mL^{-1}).



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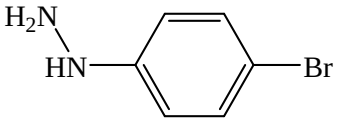
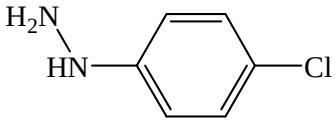
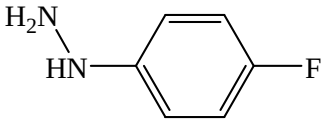


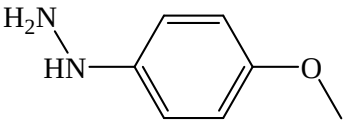
4-Bromophenylhydrazine



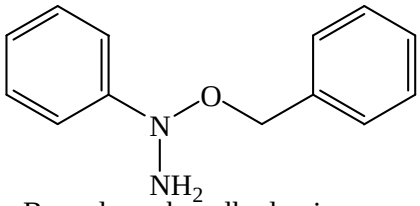
4-Chlorophenylhydrazine



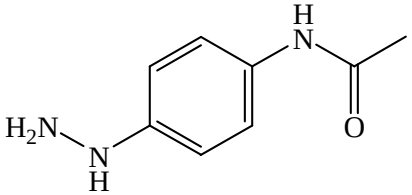
4-Fluorophenylhydrazine



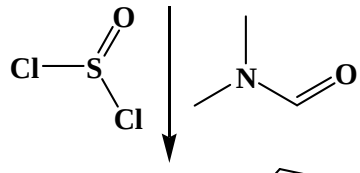
4-Methoxyphenylhydrazine

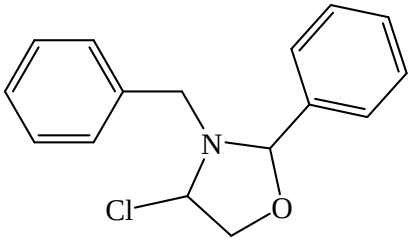


Benzyloxyphenylhydrazine

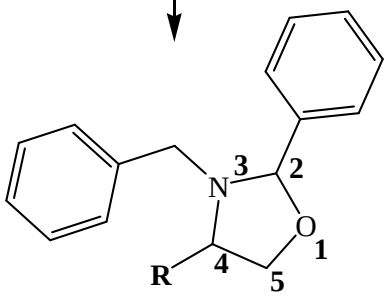


4-Acetamidophenylhydrazine





Substituted Phenylhydrazine



The potent anti-microbial activity exhibited by **6d**, **6e** may be due to the incorporation of electron donating groups like, acetamido, methoxy (at 4th position of the phenylhydrazine ring). The interesting results we observed that both electrons donating as well as electrons withdrawing groups was found to increase the anti-microbial properties, The compound **6d** and **6e** was found to possess anti-bacterial activity almost equivalent to standard drug and considerable anti-fungal activity, all the observed zone of inhibition clearly indicated in **Table 1**.

Table 1 Anti-microbial activity of the synthesized compounds (100 µg/ml)

Compounds	*In vitro* activity - zone of inhibition (MIC)								
	S.aureus	***S.epidermidis***	***M.luteus***	***B.cereus***	***E.coli***	***P.aeuriginosa***	***K.pneumoniae***	***A.niger***	***C.albicans***
6a	21(10.1)	22(9.2)	23(11.4)	19(12.7)	21(14.6)	20(16.3)	20(13.1)	20(1.2)	19(16.1)
6b	20(11.5)	20(10.6)	19(14.2)	20(10.9)	22(13.6)	21(19.1)	21(17.2)	19(10.1)	21(11.7)
6c	21(23.6)	24(20.4)	21(26.2)	19(19.2)	20(24.9)	20(19.8)	23(19.6)	21(21.1)	20(16.6)
6d	24(10.1)	26(12.2)	25(11.0)	23(13.6)	25(13.2)	23(13.1)	26(14.2)	25(9.4)	22(10.5)
6e	24(8.1)	28(10.7)	27(10.1)	22(10.6)	28(11.7)	24(11.1)	25(12.1)	26(14.1)	23(17.4)
6f	19(19.3)	18(21.2)	15(16.3)	17(15.3)	18(19.2)	18(18.6)	15(22.9)	19(26.7)	16(19.9)
Ciprofloxacin	26	30	27	24	29	24	28	-	-
Ketoconazole	-	-	-	-	-	-	-	28	26
DMF	-	-	-	-	-	-	-	-	-

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