Bioorganic & Medicinal Chemistry Letters 20 (2010) 2242–2249

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis, crystal and antibacterial studies of diversely functionalized tetrahydropyridin-4-ol

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ARTICLE INFO

Article history:
Received 12 November 2009
Revised 31 January 2010
Accepted 3 February 2010
Available online 8 February 2010

Keywords:
Tetrahydropyridine
Imidazole
Heterocycles
NMR
X-ray structure
Antibacterial activity

ABSTRACT

In an effort to expand the spectrum of antibacterial activity associated with piperidin-4-one derivatives, we have synthesized two series of 3-carboxyethyl-2,6-diphenyl-4-hydroxy-Δ^3-tetrahydropyridine derivatives bearing diversified heterocyclic and aromatic systems at the nitrogen atom through acetyl (**6**–**18**) and 2-propanoyl (**9**–**31**) linkers. Unlike acetyl derivatives, NMR spectral pattern of the propanoyl counterparts revealed the existence of pair of rotational isomers (*syn* and *anti*) in solution at room temperature due to the hindered rotation about N–CO bond. X-ray crystal studies of **9** and **24** clearly pointed out that all the compounds existed in only one form particularly, in stable *syn* form in solid state. Each of the compounds was screened for their in vitro antibacterial activity against nine human pathogenic Gram-positive strains including multiple drug resistant organisms and seven problematic Gram-negative strains. Among the various heterocycles examined here, imidazole substituted derivatives **12** and **25** exhibited antibacterial activity approaching that of Linezolid and Trovafloxacin drugs particularly against multiple resistant *Enterococcus faecium*-VanA phenotype strains.

From the beginning of 1960s, a variety of antibacterial agents have become available which are structurally assorted and proved to be efficient against a broad range of pathogenic bacteria. Despite the available agents, parasitic Gram-positive and Gram-negative bacteria continue to aggravate and kill millions of people in the subtropical regions of the world. Antibacterial resistance is now well documented for numerous pathogens and the global threat of such resistance to those established antibiotics is a serious matter under review by the World Health Organization (WHO) and many countries around the world. Of particular concern are nosocomial and community-acquired infections caused by bacterial pathogens that have become resistant to one or more antibiotics and particularly, the most challenging are methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus epidermidis* and vancomycin-resistant Enterococci (VRE). Among them, *S. aureus*, one of the opportunistic human pathogen is liable for series of infections including skin boils, bacteremia, pneumonia and endocarditis[1] while *S. epidermidis* is recognized as a pathogen affecting immunocompromised patients or those with indwelling devices.[2] Vancomycin is the consistently active agent for the treatment of serious infections due to methicillin-resistant staphylococci, but an evident unintentional consequence of its frequent use has been the emergence of high-level vancomycin-resistant enterococci (VRE) in hospitals worldwide.[3] Since the late 1990s in Republic of Korea, vancomycin-resistant enterococci turned into main pathogens responsible for endocarditis, bacteremia, and urinary and wound infections in hospital environment and the majority of vancomycin-resistant enterococcal isolates have been VanA phenotype-*vanA* genotype strains.[4] The rising incidence of VRE infections has likewise become very troublesome as they are unresponsive to the first-line antibiotics and hence is the predominant concern for clinicians and public health system. Since the approval of Linezolid in 2000, it has become the promising therapeutic option for the VRE infections[5] in patients either resistant to or unable to receive a penicillin antibiotic. Even if laboratory investigations indicate the onset of resistant mutants at a very low frequency in enterococci,[6] several instances of emergence of resistance by the increased Linezolid use have been documented in clinical isolates of enterococci viz., *Enterococcus faecalis* and *Enterococcus faecium*.[7] The frequent raise of these difficult-to-treat, multidrug-resistant VRE pathogens augment the mortality rates, as they cause very delicate and sometimes untreatable infections. Therefore, all these resistant strains complicate the treatment, and extremely affect the recovery of patients.[8] Besides these Gram-positive pathogens, various life-threatening infections caused by some Gram-negative pathogens such as *Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* and, few major respiratory tract community pathogens such as *Haemophilus influenzae* and *Moraxella catarrhalis* are also of serious concern in last few years. A striking increase in antibiotic resistance chiefly among Gram-positive bacteria has stimulated the need to screen new compounds for the development of novel antimicrobials rather than analogs of the licensed drugs in market.

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doi:10.1016/j.bmcl.2010.02.015

The importance of heterocyclic compounds, particularly nitrogen, oxygen and sulfur containing compounds has long been recognized in the field of medicinal chemistry as they possessed noticeable biological activities. Our earlier findings have shown the utility of N-functionalized 2,6-diarylpiperidin-4-one based chemical libraries of small molecules for the identification of highly active antimicrobials, antimycobacterial, potent analgesics and antipyretics.[9] In an attempt to uncover interesting antibacterial leads, recently our group has performed screens versus several clinically challenging Gram-positive and Gram-negative organisms, and noticed *N*-chloroacetyl-3-carboxyethyl-2,6-diphenyl-4-hydroxy-Δ^3-tetrahydropyridine as the potent candidate.[10]

In continuation of our earlier findings, we now focused primarily on 3-carboxyethyl-2,6-diphenyl-4-hydroxy-Δ^3-tetrahydropyridine as our core structure for the generation of library of compounds through its combination with other nitrogen, sulfur and oxygen containing heterocycles, and some aromatic ring systems by means of acetyl and 2-propanoyl linkers. Thus in this communication, we report on the initial structure–activity relationship (SAR) studies of these diversified molecules that have been investigated for their antibacterial activity against a series of human pathogenic Gram-positive and Gram-negative bacteria.

In order to study the impact of substituents about *N*-acyl system [N–$COCH_2$–*Nu*/N–COCH(CH_3)-*Nu* (*Nu*-nucleophile)] in **1** on antibacterial activity, we have prepared the respective *N*-acyl intermediates **2** and **4/5** by simple coupling of **1** with bromoacetyl chloride and 2-bromopropionyl bromide, respectively. Unlike bromoacetylation, 2-bromo propionylation resulted in the formation of two separable isomers **4** and **5**. Of the two, the isomer **4** formed as white crystal suitable for X-ray analysis. The ORTEP[11] plot of **4** (Fig. 1) demonstrate that it crystallized with two independent molecules per asymmetric unit, where the methyl group of the ethoxycarbonyl unit is disordered over two positions. Moreover, the tetrahydropyridine ring in one of the independent molecules adopts a half-chair conformation, while the other takes distorted envelope conformation. Room temperature NMR revealed that H-2 proton in **4** was deshielded significantly than H-6 (in comparison with **5**) whereas in **5**, H-6, –COC*H*Br(CH_3) and –COCHBr(CH_3) were relatively well deshielded (in comparison with **4**). X-ray and NMR studies therefore confirm that isomer **4** is in *syn* form (C=O and C-2 carbon are *syn* with each other, and hence H-2 was well-deshielded) while **5** is in *anti* form (C=O and C-2 carbon are *anti* with each other). The desired target compounds **6**–**18** and **19**–**31** (Scheme 1) were achieved by the nucleophilic substitution of the diversified nucleophiles with the respective intermediates **2** and **4** [by keeping in view of the better yield and antibacterial activity (Tables 1 and 2) of rotamer **4** than **5**, we have chosen **4** for further functionalization].

Since we have employed different heterocycles, optimization of reaction condition (with suitable base and solvent) is the decisive factor here. For six-membered cyclic amines, K_2CO_3/benzene system worked well for both the series (**6**–**9**/**19**–**22**) except in piperidin-4-one, HCl, where NEt_3/acetonitrile and crushed KOH/DMSO[12] were used, respectively, to get compounds **10** and **23**. Among the different bases used for aromatic heterocyclic amines (for compounds **11**/**24**, **12**/**25**, **13**/**26** and **14**/**27**), crushed KOH in DMSO[12] was chosen as an appropriate one as it push the reaction forward in 2–3 h at room temperature itself. Obviously, K_2CO_3/benzene was also a suitable system to achieve compounds **14** and **27** but requires longer reaction time. In the case of 5-phenyltetrazole (obtained through click reaction[13] as shown in Scheme 1A in Supplementary data), K_2CO_3/acetone was used to get **15** and **28** whereas for benzoxazol-2-thiol (achieved through the condensation of 2-aminophenol and CS_2 as depicted in Scheme 1B in Supplementary data)[14] as nucleophile, NaOH/EtOH:H_2O (1:1) and K_2CO_3/benzene were used, respectively, to obtain compounds **16** and **29**. Nucleophilic substitution reaction to accomplish compounds **17** and **18** was successful only with KO^{t}Bu/dry THF while for **30** and **31**, such strong base and dry condition were not needed instead K_2CO_3/benzene and crushed KOH/DMSO, respectively, gave satisfactory results.

Earlier, the dynamic NMR study had confirmed the existence of rotational isomers (*syn* and *anti* rotamers) at low temperatures in the case of *N*-benzimidazolylacetyl-2,6-diarylpiperidin-4-ones caused by restricted rotation about N–C=O bond.[15] However, a single set of signals characteristic for the above said compounds were

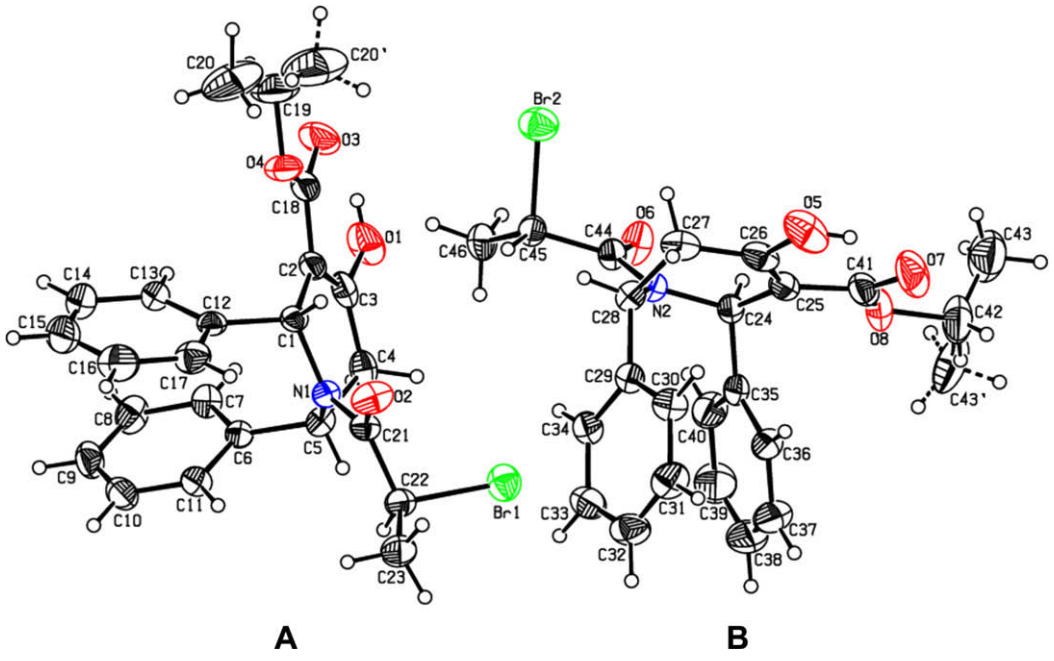
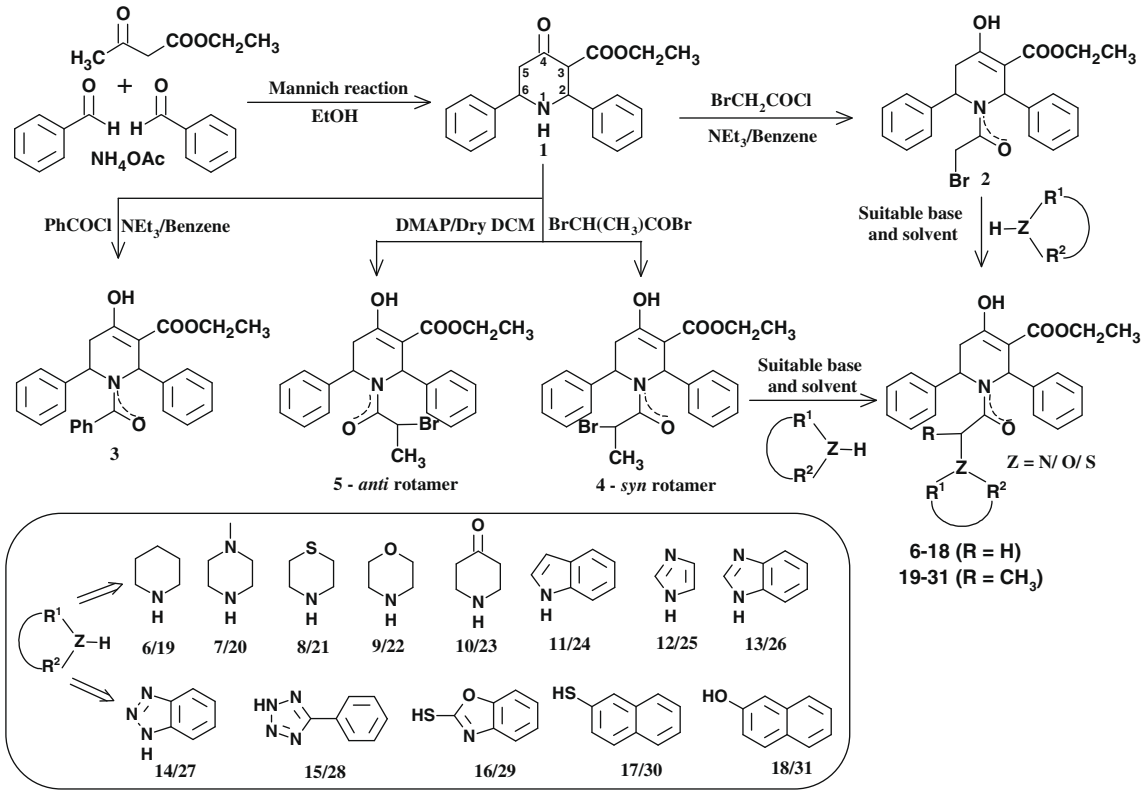


Figure 1. ORTEP plot of **4**.[11] (Compound crystallizes with two independent molecules per asymmetric unit and the methyl group of the ethoxycarbonyl unit is disordered over two positions.



Scheme 1. Synthesis of target compounds **6**–**35**.

observed at room temperature (rt) NMR because the said rotamers undergo faster interconversion on NMR time scale at rt. Likewise, the compounds **6**–**18** in the present study (having the same structural analogy about N–C=O) also furnished only one set of signals (average NMR signal) in $CDCl_3$ at room temperature NMR. The partial double bond character of the amide bond and unsaturation about C(3)–C(4) bond due to keto–enol tautomerism in **6**–**18** provide approximately a non-chair conformation for the tetrahydropyridine moiety where the ring becomes flattened at the nitrogen end with coplanarity of amide framework. This is also revealed from the broadening of benzylic proton (H-2/H-6) signals with their respective resonances in the most downfield region. As well, more deshielding of H-2 proton compared to H-6 in **6**–**18** suggests that in the preferred conformation of these compounds, H-2 proton is *syn* to carbonyl oxygen and H-6 proton is *anti* to carbonyl group. In order to confirm all these remarks, single crystal X-ray analysis was carried out for the representative compound 1-(2-morpholinoacetyl)-3-carboxyethyl-2,6-diphenyl-4-hydroxy-Δ^3-tetrahydropyridine **9** [the crystallographic data and refinement parameters of this compound are furnished in Supplementary data (Table 1)] and its ORTEP diagram is shown in Figure 2 with important bond lengths and bond angles. The X-ray data of **9** suggests that sum of the bond angles at N1 (357.9(6)°) and N2 (329.4(6)°) are in accordance with sp^2 and sp^3 hybridization, respectively. The puckering parameters[16] and the smallest displacement asymmetry parameters[17] for the morpholine and tetrahydropyridine rings are $q_2 = 0.014(3)$, 0.356(2) Å, $q_3 = -0.576(3)$, 0.291(2) Å $Q_T = 0.576(3)$, 0.460(2) Å and $\theta = 177.5(3)°$, 50.8(2)°, respectively. The morpholine moiety adopts chair conformation with atoms N2 and O5 deviating by −0.678(1) and 0.648(1) Å, respectively, from the least square plane defined by rest of the atoms in the ring and the tetrahydropyridine moiety (atoms N1/C1–C5) adopts distorted half-chair conformation. And, the dihedral angle between the two phenyl rings attached to the tetrahydropyridine moiety is about 34.5(1)° which suggests that phenyl at C5 is oriented in equatorial direction while the other at C1 is in axial orientation. As well, the observed bond angles and bond lengths about amide group (given with Fig. 2) clearly reveal coplanarity of acetyl function besides *syn* orientation of carbonyl group to H-2.

Unlike compounds **6**–**18**, ^1H and ^{13}C NMR spectra of **19**–**31** were quite complex owing to substantial peak doubling. It seemed likely that rotamers (*syn* and *anti*) of **19**–**31** exist in equilibrium in solution as a result of hindered or slow rotation of the amide C–N bond, giving rise to this doubling phenomenon. To clarify these rotational behaviors and NMR complexity, we have selected 1-[2-(1*H*-indol-1-yl)propanoyl]-3-carboxyethyl-2,6-diphenyl-4-hydroxy-Δ^3-tetrahydropyridine (**24**) as a representative compound from these set of compounds and studied extensively with the help of different NMR techniques (dynamic, one and two-dimensional NMR) and single crystal XRD.

Solution state ^1H NMR spectrum of **24** showed two distinguishable sets of signals except for aromatic protons (complex multiplet observed for aromatic protons) and hence could be unambiguously assigned based on their relative intensity, multiplicity, integral and chemical shifts values in support of 2D NMR spectral data, and also by comparing the well assigned chemical shifts of the respective intermediate rotamers (**4** and **5**). Thus, methyl protons of –$COOCH_2CH_3$ and N–$COCH(CH_3)$ groups appeared as triplet and doublet, respectively, at 0.59/1.09 ppm and 1.69/1.79 ppm while methylene protons at C-5 appeared as doublet and multiplet at 2.69 and 2.81–2.94 ppm, respectively. In the region 3.12–4.16 ppm, we have encountered another multiplet and two equal intense sextets. Here, the multiplet (centered at 4.15 ppm) could be assigned to –$COOCH_2CH_3$ of one of the rotamer whereas for the counter rotamer, these geminal protons became non-equivalent (diasteriotopic protons: –$COOCHaHbCH_3$) and hence showed two equal intense sextets centered, respectively, at 3.14 and 3.78 ppm. Similarly, pair of each singlet, triplet and quartet at

Table 1
In vitro antibacterial activities (MIC_{90} in μg/mL) of compounds **2**–**31** and, reference drugs Linezolid and Trovafloxacin against selected sensitive and resistant Gram-positive bacterial strains

Entry	Strains								
	S.A[a]	S.A[b]	S.E[c]	S.E[d]	E.F[e]	E.F[f]	E.F[g]	E.F[h]	E.F[i]
Minimum inhibitory concentration (MIC_{90}) of compounds in μg/mL									
2	>128	>128	>128	>128	>128	>128	>128	>128	128
3	>128	>128	>128	>128	>128	>128	>128	>128	>128
4	>128	128	>128	>128	>128	>128	>128	>128	128
5	>128	>128	>128	>128	>128	>128	>128	>128	>128
6	>128	>128	>128	>128	>128	>128	>128	>128	128
7	>128	>128	>128	>128	>128	>128	>128	>128	128
8	>128	>128	>128	>128	>128	>128	>128	>128	>128
9	>128	>128	>128	>128	>128	>128	>128	>128	>128
10	>128	>128	>128	>128	>128	>128	>128	>128	>128
11	>128	>128	>128	>128	>128	>128	>128	>128	>128
12	128	64	>128	>128	>128	>128	64	64	>128
13[10]	32	>128	>128	>128	64	>128	>128	>128	64
14	>128	>128	>128	128	>128	>128	>128	128	64
15	>128	>128	>128	>128	>128	>128	>128	>128	128
16	>128	>128	>128	>128	>128	>128	>128	>128	>128
17	>128	>128	>128	>128	>128	>128	>128	>128	128
18	>128	>128	>128	>128	>128	>128	>128	>128	128
19	>128	>128	>128	>128	>128	>128	>128	>128	128
20	>128	>128	>128	>128	>128	>128	>128	>128	>128
21	>128	>128	>128	>128	>128	>128	>128	>128	>128
22	>128	>128	>128	>128	>128	>128	>128	>128	128
23	>128	>128	>128	>128	>128	>128	>128	>128	>128
24	>128	>128	>128	>128	>128	>128	>128	>128	>128
25	32	64	>128	>128	>128	>128	64	128	>128
26	64	64	>128	128	>128	>128	>128	>128	>128
27	>128	>128	>128	>128	>128	>128	>128	>128	>128
28	>128	>128	>128	>128	>128	>128	>128	>128	>128
29	>128	>128	>128	>128	>128	>128	>128	>128	>128
30	>128	>128	>128	>128	>128	>128	>128	>128	>128
31	>128	>128	>128	>128	>128	>128	>128	>128	>128
X	2	2	4	8	4	4	64	2	4
Y	<0.0625	2	<0.0625	<0.0625	<0.0625	16	16	128	32

LCB—M/s LegoChem Biosciences, Inc., Daejeon, Republic of Korea (where the activity test was carried out); X—Linezolid (standard drug); Y—Trovafloxacin (standard drug).
[a] *S. aureus* (LCB0001).
[b] Methicillin-resistant *S. aureus* (LCB0002).
[c] *S. epidermidis* (LCB0003).
[d] Methicillin-resistant *S. epidermidis* (LCB0004).
[e] *E. faecalis* (LCB0005).
[f] Vancomycin-resistant *E. faecalis* Van A phenotype (LCB0006).
[g] Vancomycin and Linezolid-resistant *E. faecalis* Van A phenotype (LCB0007).
[h] Vancomycin-resistant *E. faecium* Van A phenotype (LCB0008).
[i] *E. faecium* (LCB0009).

7.01/5.89, 5.24/5.82 and 5.42/5.73 ppm are, respectively, due to H-2, H-6 and N–CO*CH*(CH_3). In order to confirm the above made assignments and to differentiate two sets of signals corresponding to each rotamer, COSY NMR was recorded. COSY spectrum exhibited strong mutual correlations between 1.09/4.16–4.10; 1.79/5.42; 2.81–2.94/5.24 ppm in one set of signals (for rotamer 1) and 0.59/3.14, 3.78; 1.69/5.73; 2.69/5.82; 3.14/3.78 ppm in other set of signals (for rotamer 2). Thus, the noticed correlations allowed for the easy identification and assignment of set of signals pertaining to each rotamer (refer Supplementary data). Here the ratio of major (signals with more intensity) to minor (signals with less intensity) rotamer was evaluated to be 60:40 on the basis of the integral value of ester methyl protons. Furthermore, exchange cross-peaks[18] observed in NOE (refer Supplementary data) also confirmed the existence of two exchangeable rotamers in solution. By taking into account the δ values of H-2 (7.01 ppm) proton in major isomer which experiences strong anisotropic deshielding effect by C=O group of propanoyl linker, it was inferred that H-2 is oriented *syn* (*cis*) to C=O group and *anti* (*trans*) to –CH(CH_3) group whereas that in minor isomer, H-2 is comparatively shielded (5.89 ppm) and thus directed *anti* (*trans*) to C=O group and *syn* (*cis*) to –CH(CH_3) group. As well, the observed vicinal coupling constant values (6.85 and 6.43 Hz) for H-6 proton in both the rotamers suggest that the tetrahydropyridine ring is not in the normal chair conformation. Furthermore, to check the dynamics of the interconversion between the two rotamers, a variable temperature NMR study was carried out in $CDCl_3$ over the temperature range 293–333 K (20–60 °C). Figure 3 shows that upon increasing the temperature, line broadening occurs up to 313 K and finally, each couple of signals corresponding to major and minor rotamers, almost coalesces to one resonance (average NMR) at 333 K. Dynamic NMR study confirms our hypothesis that at room temperature, slow exchange process occurs between the rotamers when compared to NMR time scale (and thus gave two sets of signals) while at high temperature, fast exchange process leads to broadening and coalescence of the each set of signals in **24**.

Observations of two sets of signals in ^{13}C NMR was also in agreement with the existence of both *syn* and *anti* rotamers in solution (refer Supplementary data). Once the resonances of the respective protons were established, analysis of the HSQC spectra allowed for the distinction between the chemical shift values of the carbons of each rotamer and their respective assignments. Unequivocal assignment of important quaternary carbons such as C-3, amide and ester carbonyl carbon for both the series of signals was derived from HMBC experiment because it verified the existence of two and three-bond connectivities of the said carbons

Table 2
In vitro antibacterial activities (MIC_{90} in μg/mL) of compounds **2**–**31** and, reference drugs Linezolid and Trovafloxacin against selected Gram-negative bacterial strains

Entry	Strains						
	E.C[a]	E.C[b]	E.C[c]	P.A[d]	K.P[e]	H.I[f]	M.C[g]
Minimum inhibitory concentration (MIC_{90}) of compounds in μg/mL							
2	>128	>128	>128	>128	>128	>128	>128
3	>128	>128	64	>128	>128	>128	>128
4	>128	>128	>128	128	>128	128	>128
5	>128	>128	>128	>128	>128	>128	>128
6	>128	>128	64	>128	>128	>128	32
7	>128	>128	>128	>128	>128	>128	32
8	>128	>128	>128	>128	>128	>128	64
9	>128	>128	>128	>128	>128	>128	64
10	>128	>128	>128	>128	>128	>128	64
11	>128	>128	>128	>128	>128	>128	32
12	>128	>128	64	>128	>128	>128	64
13[10]	>128	>128	>128	>128	>128	>128	32
14	>128	>128	>128	>128	>128	>128	>128
15	>128	>128	>128	>128	>128	>128	>128
16	>128	>128	>128	>128	>128	>128	>128
17	>128	>128	>128	>128	>128	>128	>128
18	>128	>128	>128	>128	>128	>128	>128
19	>128	>128	>128	>128	>128	>128	64
20	>128	>128	>128	>128	>128	>128	64
21	>128	>128	>128	>128	>128	>128	>128
22	>128	>128	>128	>128	>128	>128	>128
23	>128	>128	128	>128	>128	>128	>128
24	>128	>128	128	>128	>128	>128	>128
25	>128	>128	64	128	128	>128	64
26	>128	>128	>128	>128	>128	>128	>128
27	>128	>128	>128	>128	>128	>128	>128
28	>128	>128	>128	>128	>128	>128	>128
29	>128	>128	>128	>128	>128	>128	>128
30	>128	>128	>128	>128	>128	>128	>128
31	>128	>128	>128	>128	>128	>128	>128
X	>128	16	128	>128	>128	16	4
Y	<0.0625	<0.0625	<0.0625	0.5	0.125	<0.0625	<0.0625

LCB—M/s LegoChem Biosciences, Inc., Daejeon, Republic of Korea (where the activity test was carried out); X—Linezolid (standard drug); Y—Trovafloxacin (standard drug).
[a] *E. coli* (LCB0010).
[b] *E. coli* (LCB0011).
[c] *E. coli* (LCB0012).
[d] *P. aeruginosa* (LCB0013).
[e] *K. pneumoniae* (LCB0014).
[f] *H. influenzae* (LCB0015).
[g] *M. caterrhalis* (LCB0016).

with the adjacent protons (refer Supplementary data for HMBC correlations-Table 2).

In order to examine the conformational preference in solid state, crystal structure was studied for 1-[2-(1*H*-indol-1-yl)propanoyl]-3-carboxyethyl-2,6-diphenyl-4-hydroxy-Δ^3-tetrahydropyridine (**24**), which fortunately obtained as a single crystal by slow evaporation of a solution in ethanol. The crystallographic data and refinement parameters of **24** are furnished in Supplementary data (Table 3). ORTEP plot of asymmetric unit of **24** shown in Figure 4 (with important bond lengths and bond angles) obviously displays that the crystal structure of **24** is in stable *syn* form (C=O *syn* to H-2). The sum of the bond angles at N1 [359.4(3)°] (revealed from the shortening of C(6)–N(1) bond length to 1.355 Å by the partial double bond character of N1–C6=O1) and N2 [359.8(3)°] are in accordance with sp^2 hybridization. The torsion angles C11–C10–C15–N2 [−179.9(2)] and C9–C10–C15–C14 [179.3(2)] suggest that the indole moiety is planar. The tetrahydropyridine ring (atoms N1/C1–C5) in the present structure adopts boat conformation with atoms C2 and C5 deviating by 0.495(1) Å and 0.500(1) Å, respectively, from the least square plane defined by rest of the atoms in the ring. The puckering parameters[16] and the smallest displacement asymmetry parameters[17] for tetrahydropyridine ring are $q_2 = 0.575(2)$ Å, $q_3 = -0.001(2)$ Å, $Q_T = 0.575(2)$ Å and $\theta = 90.1(2)°$. And, unsaturation about C(3)–C(4) bond is established from the decrease in its bond length (1.34 Å) compared to other single bonds (≈1.5 Å) in the ring system whereas the coplanarity of 2-propanoyl moiety with C1–N1–C5 plane is noticed from the observed bond parameters (given with Fig. 4). Moreover, the two phenyl rings attached to the tetrahydropyridine moiety are nearly perpendicular to each other (i.e., to relieve $A^{1,3}$ strain by the coplanar carbonyl group, phenyl groups are flipped from equatorial to axial orientation) with the dihedral angle of 88.3(1)°. Thus, according to the observed NMR and X-ray data, two boat conformers (which are in equilibrium with each other in solution) are proposed for *syn* and *anti* rotamers of compound **24** as shown in Figure 5.

In view of the similarity of the ^1H and ^{13}C NMR spectra for the tetrahydropyridine moiety in amide **24** with other derivatives (**19**–**23** and **25**–**31**), the complete assignment of the individual protons and carbons for both the rotamers has been made. Finally, NMR (dynamic, one and two-dimensional NMR) studies concluded the existence of inseparable rotamers in equilibrium in solution slowly interconverting to each other on NMR time scale at room temperature, conversely, the single crystal X-ray analysis confirmed the existence of only one form (i.e., stable *syn* form) in solid state.

In vitro antibacterial testing of the series of derivatives was carried out by using National Committee for Clinical Laboratory Standards (NCCLS)[19] assay against a panel of both susceptible and resistant Gram-positive bacterial strains viz. methicillin-sensitive *S. aureus/S. epidermidis*, methicillin-resistant *S. aureus/S. epidermidis*, *E. faecalis*, *E. faecium*, vancomycin-resistant *E. faecalis*/*E. faecium* and vancomycin and Linezolid-resistant *E. faecalis*. The observed MIC_{90} (MIC_{90} is the minimum concentration of an antibacterial

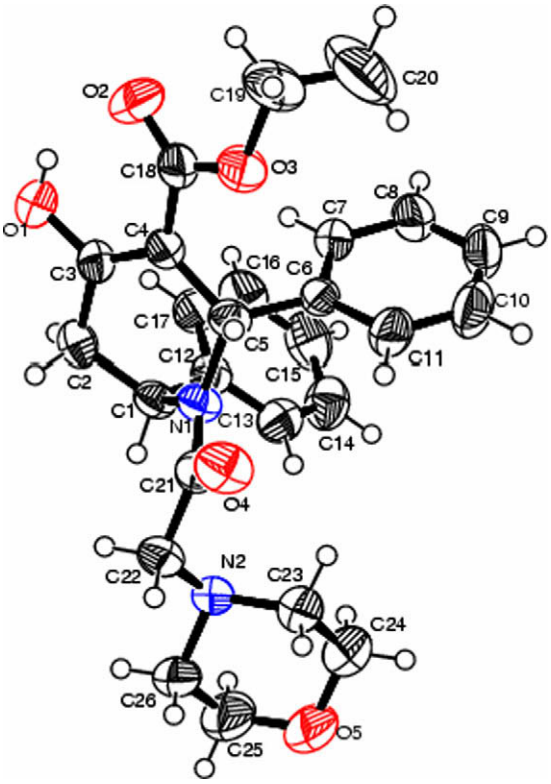


Figure 2. ORTEP plot of **9**. The important bond lengths (Å): C(1)–N(1) = 1.470(3); C(5)–N(1) = 1.476(3); C(21)–N(1) = 1.356(3); C(21)–O(4) = 1.218(3); C(21)–C(22) = 1.520(3); C(2)–C(3) = 1.484(3); C(3)–C(4) = 1.346(3); C(4)–C(5) = 1.507(3). The important bond angles (°): N(2)–C(22)–C(21) = 114.4(19); O(4)–C(21)–N(1) = 121.4(2); N(1)–C(21)–C(22) = 120.2(2); N(1)–C(5)–C(6) = 113.3(18); C(1)–N(1)–C(5) = 116.8(17); C(21)–N(1)–C(5) = 117.1(19); C(21)–N(1)–C(1) = 123.9(18).

agent required to inhibit the growth of 90% of organisms) values of the test compounds are reproduced in Table 1 along with the standard drugs Linezolid and Trovafloxacin. Test compounds were prepared at an initial concentration of 128 μg/mL in DMSO and serially diluted resulting in twofold dilution. Here DMSO and growth medium served as growth control and sterility control, respectively.

A preliminary assay data (Table 1) showed that the compounds bearing six-membered saturated cyclic amines were not efficient in inhibiting the growth of all the tested strains except **6** and **7**, which exhibited inhibition at 128 μg/mL against *E. faecium*. Replacement of the said amines by aromatic amines has shown moderate improvement in their activity. Though compound **11** bearing indole moiety is inactive against all the strains under study, its replacement by imidazole gave compound **12** that showed better inhibitory potency at a MIC of 64 μg/mL against methicillin-resistant *S. aureus*, vancomycin-resistant *E. faecium*-VanA phenotype, and vancomycin and Linezolid-resistant *E. faecalis*-VanA phenotype. Here, the effectiveness of compound **12** against vancomycin-resistant *E. faecium*-VanA was found to be doubled to that of Trovafloxacin drug while against vancomycin and Linezolid-resistant *E. faecalis*-VanA, an equal potency was noted to that of Linezolid. Similarly, the corresponding benzotriazole derivative **14** showed same potency to that of Travofloxacin against vancomycin-resistant *E. faecium*-VanA while against *E. faecium* and methicillin-resistant *S. epidermidis*, it has registered MIC at 64 and 128 μg/mL, respectively. On the other hand, the derivatives **15**–**18** were almost inactive towards all the pathogens with the exception of *E. facium* against which compounds **15**, **17** and **18** bearing, respectively, 5-phenyltetrazole, 2-naphthalenethiol and 2-naphthol showed inhibition at a minimum concentration of 128 μg/mL. To investigate the effect of *C*-methyl group in the *N*-acetyl functionality on the antibacterial activity, we have synthesized the other series of compounds (**19**–**31**) and screened against the same panel of Gram-positive strains. However, this second series of compounds also displayed the same trend in the antibacterial profile. The saturated cyclic amine derivatives **19**–**23** were inactive up to a maximum concentration of 128 μg/mL except **19** and **22**, for which growth inhibition was noticed at a minimum concentration of 128 μg/mL against *E. facium*. Switching over from

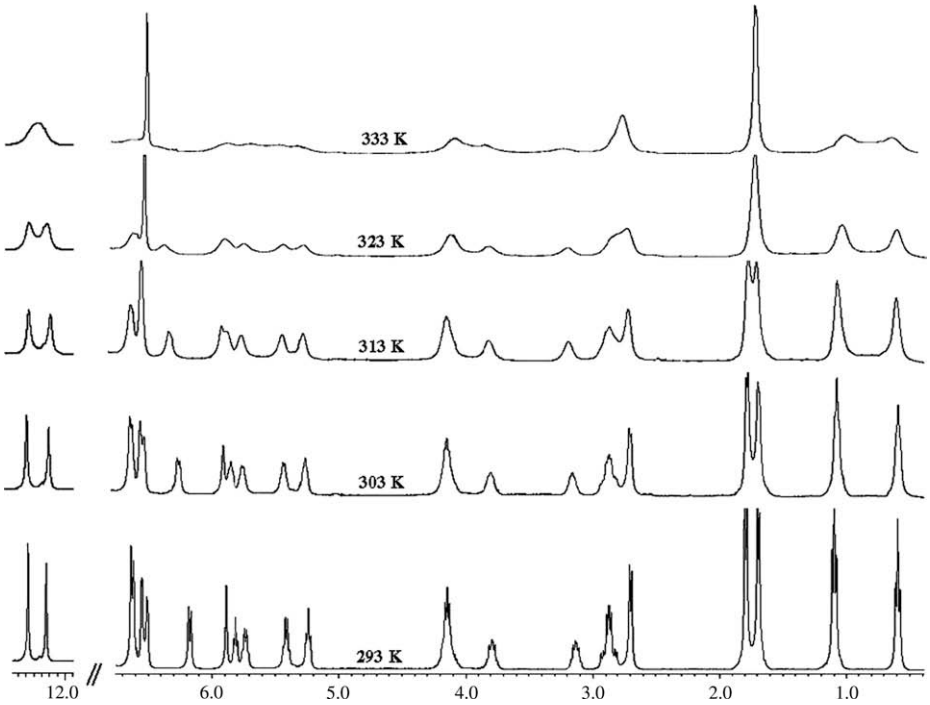


Figure 3. Temperature dependant NMR spectra of **24**.

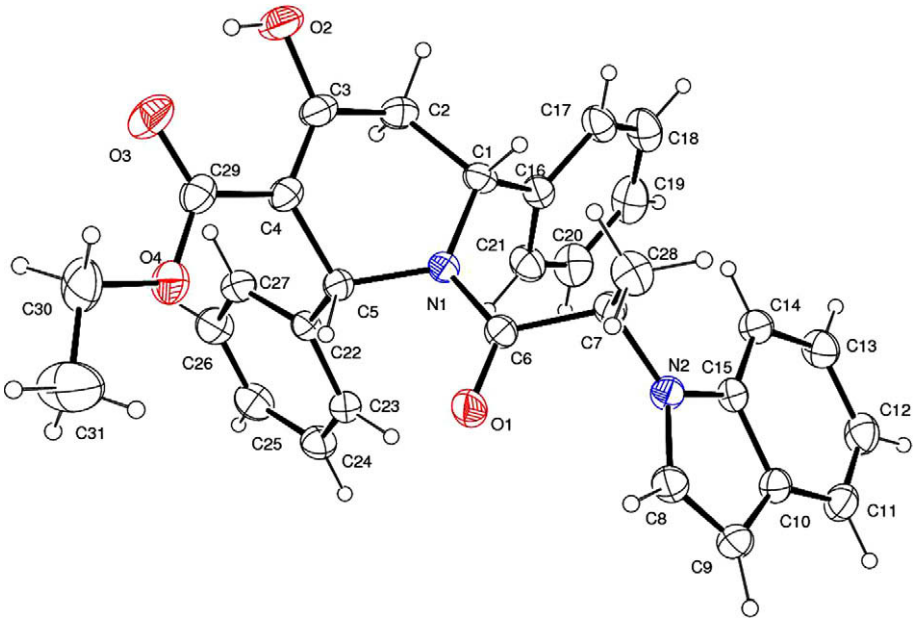


Figure 4. ORTEP plot of **24**. The important bond lengths (Å): C(1)–N(1) = 1.479(19); C(5)–N(1) = 1.490(2); C(6)–N(1) = 1.355(2); C(6)–O(1) = 1.219(19); C(6)–C(7) = 1.536(2); C(2)–C(3) = 1.478(3); C(3)–C(4) = 1.336(2); C(7)–C(28) = 1.521(2); The important bond angles (°): N(2)–C(7)–C(6) = 110.3(13); O(1)–C(6)–N(1) = 122.3(15); N(1)–C(6)–C(7) = 119.4(14); C(6)–N(1)–C(1) = 122.9(13); C(6)–N(1)–C(5) = 116.5(13); C(1)–N(1)–C(5) = 119.9(13).

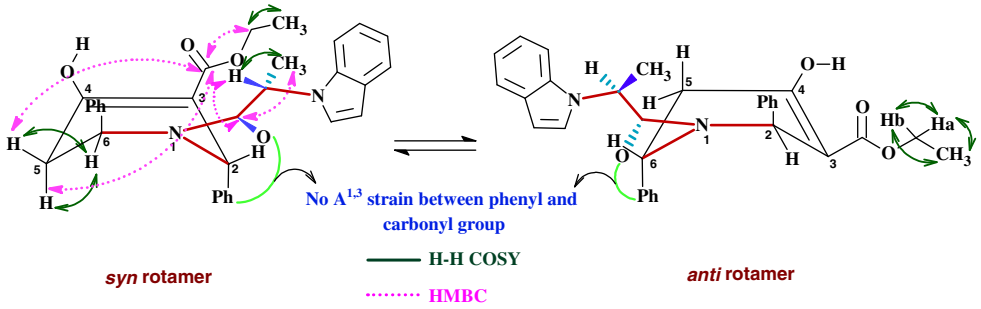


Figure 5. *syn* and *anti* forms of compound **24** in equilibrium in solution. Selected H–H COSY and HMBC correlations are shown (both *syn* and *anti* rotamers exhibited the same kind of correlations while the additional COSY correlations observed in *anti* rotamer are shown separately). Bonds in red color highlight the coplanarity of 2-propanoyl system with C2–N1–C6 plane.

saturated cyclic amines to aromatic amines, imidazole (**25**) and benzimidazole (**26**) only exhibited marked activity against some of the sensitive and resistant Gram-positive pathogens while their replacement by either of the other heterocyclic amines or naphthalene derivatives led to complete loss of antibacterial potency. Compared to **12** (*N*-acetyl derivative), Compound **25** [*N*-2-(propanoyl) derivative with imidazole nucleus] exhibited twofold increased inhibitory potency against *S. aureus* and onefold decreased potency against vancomycin-resistant *E. faecium*. Nonetheless, potency of **25** against Vancomycin-resistant *E. faecium* is equivalent to that of Trovafloxacin. Substitution of benzimidazole in place of imidazole in **25** (i.e., compound **26**) showed inhibitions at 64 and 128 μg/mL against *S. aureus*/MR *S. aureus and* MR *S. epidermidis*, respectively, while against rest of the strains, it was almost inactive.

Besides these antibacterial screening against a panel of Gram-positive organisms, they were also examined towards a group of pathogenic Gram-negative bacteria such as three different *E. coli* strains (LCB0010–LCB0012), *P. aeruginosa*, *K. pneumoniae*, *H. influenzae* and *M. catarrhalis*. The observed MIC_{90}'s are reproduced in Table 2.

It is interesting to note that even if substitution of various heterocycles or homocycles results in the devoid of antibacterial activity against two types of *E. coli* strains LCB0010 and LCB0011, compounds **3**, **6**, **12**, **23**–**24** registered an attractive and comparable inhibitory power to Linezolid drug against *E. coli* LCB0012. *N*-Benzoyl derivative **3** registered MIC at 64 μg/mL while the incorporation of piperidinoacetyl (compound **6**) and imidazolylacetyl (compound **12**) in lieu of benzoyl group retained the same potency. But the other kind of heterocycle modification leads to the loss of activity. Moreover, compounds **10** and **11**, which were inactive against *E. coli* LCB0012, became active with MIC 128 μg/mL through the introduction of methyl group in the *N*-acetyl moiety (i.e., compounds **23** and **24**, respectively, with *N*-2-propanoyl functionality) but the same kind of modification in **12** (compound **25**) did not enhance the potency. A striking observation from the results in Table 2 is that compounds **23**/**24** and **3**/**6**/**12**/**25** demonstrated, respectively, equivalent and onefold improved inhibitory power than Linezolid. Apart from **4**/**25**, **25**, **4** against *P. aeruginosa*, *K. pneumoniae* and *H. influenzae* (MIC at 128 μg/mL) respectively, all other derivatives were inactive up to a concentration of 128 μg/mL. Against *M. caterrhalis*, most of the *N*-acetyl derivatives (**6**–**13** with MIC 32–64 μg/mL) seems better than the corresponding *N*-(2-propanoyl) derivatives (**19**, **20**, **25** with MIC 64 μg/mL). Thus substitution of either piperidine (**6**) or *N*-methylpiperazine (**7**) system in place of bromine in **2** recorded about twofold improved antibacterial activity (32 μg/mL) while their replacement

by either thiomorpholine (**8**) or morpholine (**9**) or piperidin-4-one (**10**) resulted in onefold (64 μg/mL) enhancement. Similarly, introduction of aromatic heterocycles such as indole (**11**) and imidazole (**12**) also recorded MIC, respectively, at 32 and 64 μg/mL.

In conclusion, we have achieved heterocyclic and homocyclic substituted acetyl (**6**–**18**) and propanoyl (**19**–**31**) derivatives of tetrahydropyridin-4-ol in moderate to good yields and studied extensively through various NMR techniques. The cumulative NMR and X-ray analysis concluded that all the compounds **6**–**31** were in stable *syn* form in solid state whereas propanoyl derivatives **19**–**31** exist in two inseparable rotameric conformations (*syn* and *anti*) in solution. Antibacterial screening study against clinically relevant Gram-positive and Gram-negative pathogens revealed that acetyl derivatives were comparatively better than 2-propanoyl analogues. Generally amide functional group has different steric, electronic and lipophilic characteristics depending upon the substituent in it. Moreover, the possible conformation of the acetyl derivatives may allow for the facile penetration of the cell wall of the bacteria while for the corresponding 2-propanoyl derivatives, such physical process may be lacking owing to their assumed conformation. As well, among the various heterocycles tested here, imidazole derivatives (**12** and **25**) are emerged as potent compounds as they showed promising and comparable activity to that of the standard Linezolid and Trovafloxacin drugs particularly against multiple resistant enterococci VanA phenotype strains, thereby considered for further structural optimization.

Acknowledgments

This research work was supported by the grant from second stage of BK21 program. We are also grateful to M/s LegoChem Biosciences, Inc., Daejeon, Republic of Korea for their help in conducting antibacterial screening tests and Dr. D. Gayathri, Institute of Structural Biology and Biophysics-2, Germany for her useful discussions about crystal structure of the compounds.

Supplementary data

Detailed experimental procedure and characterization data for all the compounds along with $^{1}H/^{13}C$ NMR, COSY, NOESY, HSQC and HMBC spectra for the representative compound **24**. The crystallographic data of **9** and **24** have been deposited at Cambridge Crystallography Data Center (CCDC Nos. 685679 and 748022, respectively). Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.02.015.

References and notes

1. (a) Chan, W. C.; Coyle, B. J.; Williams, P. *J. Med. Chem.* **2004**, *47*, 4633; (b) Kloos, W. E. Staphylococcus. In *Topley and Wilson's Microbiology and Microbial Infections*; Collier, L., Balows, A., Sussman, M., Eds., 9th ed.; Systematic Bacteriology; Edward Arnold: London, 1998; Vol. 2, pp 577–632.
2. (a) Raad, I.; Alrahwan, A.; Rolston, K. *Clin. Infect. Dis.* **1998**, *26*, 1182; (b) Gotz, F. *Mol. Microbiol.* **2002**, *43*, 1367.
3. (a) Levy, S. B.; Marshell, B. *Nat. Med.* **2004**, *10*, S122; (b) Wagenlehner, F. M. E.; Naber, K. G. *Int. J. Antimicrob. Agents* **2004**, *24S*, S39; (c) Mitchell, M. O. *Anti-Infect. Agents Med. Chem.* **2007**, *6*, 243.
4. (a) Cheong, H. J.; Kim, W. J. *Korean J. Nosocom. Infect. Control* **1998**, *3*, 57; (b) Hong, W. P.; Kim, M.; Song, J. W.; Kim, S. H.; Shin, J. H.; Seo, S. P.; Yang, D. W. *Korean J. Clin. Pathol.* **1998**, *18*, 379; (c) Cheong, H. J.; Park, C. W.; Eom, J. S.; Ha, K. I.; Shun, J. H.; Kim, C. M.; Lee, H. S.; Park, Y. H.; Kim, W. J.; Park, S. C. *Korean J. Infect. Dis.* **2002**, *34*, 285.
5. (a) Murray, B. E. *N. Engl. J. Med.* **2000**, *342*, 710; (b) Linden, P. K.; Moellering, R. C.; Wood, C. A.; Rehm, S. J.; Flaherty, J.; Bompart, F.; Talbot, G. H. *Clin. Infect. Dis.* **2001**, *33*, 1816; (c) Birmingham, M. C.; Rayner, C. R.; Meagher, A. K.; Flavin, S. M.; Batts, D. H.; Schentag, J. J. *Clin. Infect. Dis.* **2003**, *36*, 159.
6. Prystowsky, J.; Siddiqui, F.; Chosay, J.; Shinabarger, D. L.; Millichap, J.; Peterson, L. R.; Noskin, G. A. *Antimicrob. Agents Chemother.* **2001**, *45*, 2154.
7. (a) Dibo, I.; Pillai, S. K.; Gold, H. S.; Baer, M. R.; Wetzler, M.; Slack, J. L.; Hazamy, P. A.; Ball, D.; Hsiao, C. B.; McCarthy, P. L., Jr.; Segal, B. H. *J. Clin. Microbiol.* **2004**, *42*, 1843; (b) Halle, E.; Padberg, J.; Rosseau, S.; Klare, I.; Werner, G.; Witte, W. *Infection* **2004**, *32*, 182; (c) Johnson, A. P.; Tysall, L.; Stockdale, M. V.; Woodford, N.; Kaufmann, M. E.; Warner, M.; Livermore, D. M.; Asboth, F.; Allerberger, F. J. *Eur. J. Clin. Microbiol. Infect. Dis.* **2002**, *21*, 751; (d) Lobritz, M.; Hutton-Thomas, R.; Marshall, S.; Rice, L. B. *Antimicrob. Agents Chemother.* **2003**, *47*, 3318; (e) Marshall, S. H.; Donskey, C. J.; Hutton-Thomas, R.; Salata, R. A.; Rice, L. B. *Antimicrob. Agents Chemother.* **2002**, *46*, 3334.
8. (a) Bjarnsholt, T.; Givskov, M. *Curr. Infect. Dis. Rep.* **2008**, *10*, 22; (b) Otto, M. *Curr. Top. Microbiol. Immunol.* **2008**, *322*, 207; (c) Sjölund, M.; Tano, E.; Blaser, M. J.; Andersson, D. I.; Engstrand, L. *Emerg. Infect. Dis.* **2005**, *11*, 1389.
9. (a) Aridoss, G.; Amirthaganesan, S.; Kim, M. S.; Kim, J. T.; Jeong, Y. T. *Eur. J. Med. Chem.* **2009**, *44*, 4199; (b) Aridoss, G.; Parthiban, P.; Ramachandran, R.; Prakash, M.; Kabilan, S.; Jeong, Y. T. *Eur. J. Med. Chem.* **2009**, *44*, 577; (c) Aridoss, G.; Balasubramanian, S.; Parthiban, P.; Ramachandran, R.; Kabilan, S. *Med. Chem. Res.* **2007**, *16*, 188; (d) Aridoss, G.; Parthiban, P.; Kabilan, S. *Eur. J. Med. Chem.* **2007**, *42*, 851; (e) Aridoss, G.; Balasubramanian, S.; Parthiban, P.; Kabilan, S. *Eur. J. Med. Chem.* **2006**, *41*, 268; (f) Balasubramanian, S.; Aridoss, G.; Parthiban, P.; Kabilan, S. *Biol. Pharm. Bull.* **2006**, *29*, 125; (g) Balasubramanian, S.; Ramalingan, C.; Aridoss, G.; Kabilan, S. *Eur. J. Med. Chem.* **2005**, *40*, 694.
10. Aridoss, G.; Amirthaganesan, S.; Ashok Kumar, N.; Kim, J. T.; Lim, K. T.; Kabilan, S.; Jeong, Y. T. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 6542.
11. Aridoss, G.; Gayathri, D.; Velmurugan, D.; Kim, M. S.; Jeong, Y. T. *Acta Crystallogr. Sect. E* **2009**, *65*, o1708.
12. Wilson, Z. E.; Heapy, A. M.; Brimble, M. A. *Tetrahedron* **2007**, *63*, 5379.
13. Tamoto, N.; Adachi, C.; Nagai, K. *Chem. Mater.* **1997**, *9*, 1077.
14. Liu, K. G.; Lo, J. R.; Comery, T. A.; Zhang, G. M.; Zhang, J. Y.; Kowal, D. M.; Smith, D. L.; Di, L.; Kerns, E. H.; Schechter, L. E.; Robichaud, A. J. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1115.
15. Aridoss, G.; Amirthaganesan, S.; Kim, M. S.; Cho, B. G.; Lim, K. T.; Jeong, Y. T. *Arkivoc* **2008**, 133.
16. Cremer, D.; Pople, J. A. *J. Am. Chem. Soc.* **1975**, *97*, 1354.
17. Nardelli, M. *Acta Crystallogr. Sect. C* **1983**, *39*, 1141.
18. Marakos, P.; Pouli, N.; Garoufalias, S. P.; Mikros, E. *J. Mol. Struct.* **2003**, *650*, 213.
19. National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 5th ed. Approved Standard. NCCLS Document M7-A5, National Committee for Clinical Laboratory Standards, Villanova, PA, 2000.