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Synthesis of substituted-amidine derivatives of avibactam and synergistic antibacterial activity with meropenem

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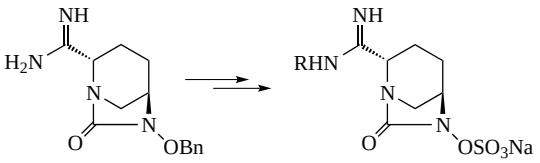
New avibactam derivatives in which amide moiety is replaced by substituted amidine groupings were obtained in several steps, the key step having involved the conversion of 2-positioned cyano group of the 1,6-diazabicyclo[3.2.1]-octane framework into the amidine one. Synergistic antibacterial activity of the new compounds in combination with meropenem infers the agonistic effect of these derivatives against β-lactamases.



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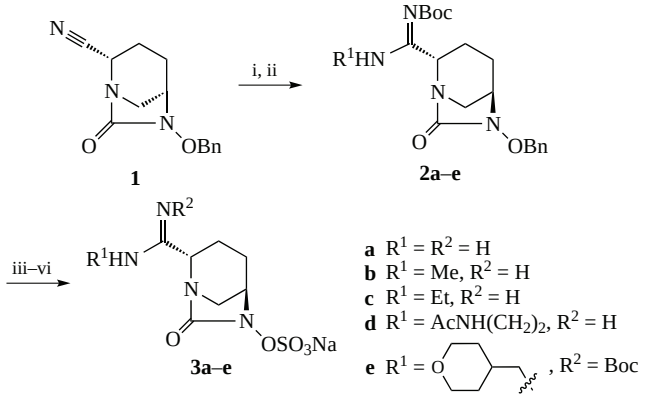
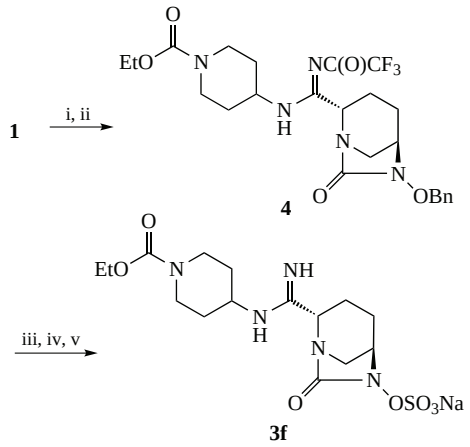
Successful employment of any therapeutic agent is hindered by the evolution of resistance.[1] Antibacterial resistance against sulfonamides[1–3] followed by the discovery of penicillinase[1] suggests that the process of resistance is inevitable. As a result, diverse classes of antibacterial agents[2,4–8] have been introduced over the years to cope with the antibacterial resistance. β-Lactams (BLs) disrupt the biosynthesis of bacterial cell wall by binding the active site of a set of serine PBPs (commonly called as penicillin binding proteins), which are responsible for the crosslinking of glycopeptide chain.[9] Bacterial strains adapted four different ways[2] of action to overcome the agonistic effect of antibacterial agents. In addition to others, modification or degradation of antibiotics is the most common strategy adopted by the bacteria against aminoglycosides, chloramphenicol and β-lactams.[8] More than 1300 β-lactamases[10] have been identified so far which have been classified into four groups by the Ambler system.[11] Development of the β-lactamase inhibitors (BLIs)[11,12] is therefore a strategy for the combination therapy[13,14] to restore therapeutic activity of existing antibiotics.

A number of small molecules have been recognized as BLIs with clavulanic acid[15] being the first one approved for clinical use[16] followed by tazobactam.[6] Non-BLIs of class A and C β-lactamases emerged when the 1,6-diazabicyclo[3.2.1]octane (DBO) scaffold was recognized as the substitute of four membered β-lactam ring.[6] Avibactam, a non-BLI derived from DBO scaffold, has been found effective inhibitor against extended spectrum β-lactamases (ESBLs), carbapenemases and multidrug resistant[17] (MDR) *Enterobacteriaceae* as well as *Pseudomonas aeruginosa*[18–20] in combination with ceftazidime.[21] Recently a handful number of avibactam derivatives has been synthesized, and a few of them are either approved or passing through phase I or phase III clinical trials.[6,13,10,22] Despite the development of DBO derivatives and others alike,[6] the process of resistance still needs new inhibitors to cope with future challenges. As part of our efforts towards the development of new BLIs,[23,24] we herein successfully synthesized a series of new substituted-amidine derivatives of avibactam and hereby report their antibacterial activities alone, and in combination with existing β-lactam (meropenem).

For the synthesis of the target compounds (Scheme 1), the starting DBO cyano derivative **1** was subjected to amidination reaction by previously described method.[23–26] The attempted next benzyl deprotection of NH-unprotected amidine derivatives was unsusscessful. Therefore, amidine intermediates were Boc-protected to afford compounds **2a**–**e** in 44 to 70% yield. Debenzylation of compounds **2a**–**e** was accomplished by heterogeneous hydrogenation, and the intermediate hydroxy



Scheme 1 *Reagents and conditions*: i, $AlMe_3$ or TMSOTf, NH_4Cl or amine-$R_{b–e}$, 0 → 20 °C, 16 h, 44–61%; ii, Boc_2O, TEA, CH_2Cl_2, room temperature, 48 h, 44–70%; iii, H_2, Pd/C (wet), MeOH or EtOAc, 16 h, room temperature, 66–94%; iv, SO_3–pyridine, pyridine or SO_3–NMe_3, TEA/MeOH, room temperature, 16 h, 74–90%; v (for compounds **3a**–**d**), TFA, CH_2Cl_2, 0 °C, 3.5 h or formic acid, 0 °C, 30 h, Dowex-50wx Na^+, 15–36%; vi (for compound **3e**), Dowex-50wx Na^+, 78%.

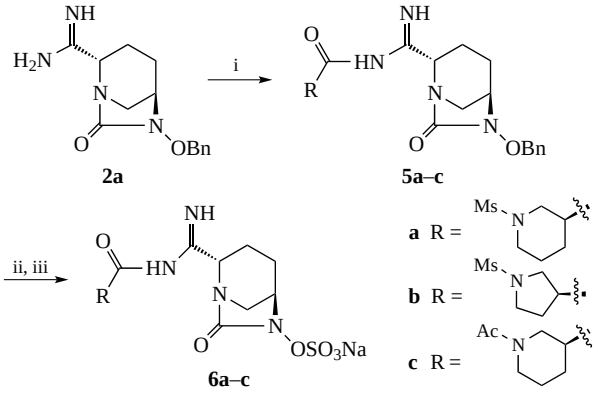


Scheme 2 *Reagents and conditions*: i, ethyl 4-aminopiperidine-1-carboxylate, TMSOTf, THF, 0 → 20 °C, 3.5 days, 26%; ii, $(CF_3CO)_2O$, TEA, CH_2Cl_2, 0 → 20 °C, 4 h, 36%; iii, H_2, Pd/C (wet), EtOAc, room temperature, 16 h, 61%; iv, SO_3–pyridine, pyridine, room temperature, 16 h, 78%; v, Na_2CO_3, $MeOH/H_2O$, room temperature, 2 h, Diaion HP-20, 10%.

products were then reacted with SO_3 as pyridine or NMe_3 salt. Subsequently, Boc groups were removed by treatment with triflouracetic acid (TFA) (except for **2e** since the deprotected product was unstable). These crude products were purified by ion exchange column filled with Dowex-50wx Na^+, and final lyophilization gave compounds **3a–e** as sodium salts.

For the synthesis of analogous compound **3f** the parallel protocol (Scheme 2) was followed. In this case, compound **1** was reacted with ethyl 4-aminopiperidine-1-carboxylate in the presence of TMSOTf in THF to obtain amidine derivative **4**. Following, amidine group in **4** was TFA-protected by reacting it with trifluoroacetic anhydride in the presence of triethylamine. The intermediate compound was debenzylated and treated with SO_3–pyridine complex followed by TFA deprotection with Na_2CO_3. The crude product was purified by Diaion HP-20 resin to afford the final compound **3f** in 10% yield.

Synthesis of compounds **6a–c** starting from intermediate **2a** was accomplished (Scheme 3) by coupling the organic acids at NH_2 grouping of amidine **2a** to form key intermediates **5a–c**.[18] Compounds **5a–c** were then debenzylated and converted into the corresponding sulfuric salts **6a–c** in moderate to excellent yields by following the procedures analogous to Schemes 1 and 2.



Scheme 3 *Reagents and conditions:* i, RC(O)OH, HATU/DIPEA, DMF, room temperature, 16–24 h, 40–81%; ii, H_2, Pd/C (wet), THF/EtOAc, TEA, room temperature, 16 h, 63–85%; iii, SO_3–pyridine/SO_3–NMe_3, pyridine or TEA, THF/H_2O, room temperature, 16 h, Dowex-50wx Na^+, 25–97%.

The new substituted-amidine derivatives of avibactam **3a–f** and **6a–c** were tested for their *in vitro* antibacterial activities against ten bacterial strains with variable β-lactamases (indicated in parenthesis, Table 1), individually, as well as in combination with meropenem (MER). Minimum inhibitory concentration (MIC) values for meropenem alone are also determined[27] in order to evaluate the synergistic effect of synthesized compounds. It can be observed that all these compounds and avibactam are not antibacterial in action (MIC, >64 mg dm^{-3}) when used alone without β-lactam. However, the antibacterial activity of the β-lactam meropenem is enhanced upon combining with compounds **3a–f**, **6a–c** and avibactam, individually. In general, all newly synthesized compounds enhanced potency of meropenem against all tested species with MIC values ranging from <0.125 to 2 mg dm^{-3} (compared to control MER MIC of 4 mg dm^{-3} in most cases). Nonetheless, compound **3c** failed to minimize the MIC of the standard antibiotic (MER) against *E. cloacae* clinical isolate. It can be noted that compounds **6a**,**b** showed highest activity against most of the ten test bacterial strains with MIC value of <0.125 mg dm^{-3} in comparison to MER alone (MIC, 4 mg dm^{-3}). Compounds **6a**,**b** were potent against *A. baumannii* clinical isolate (MIC, 0.25 mg dm^{-3}) and *A. baumannii* 19606 (MIC, 0.5 mg dm^{-3}), and also were the most effective against *P. aeruginosa* 9027 exhibiting MIC value of <0.125 mg dm^{-3}. Comparing the data, it can be concluded that clinical isolates

Table 1 Antibacterial activity of avibactam and compounds **3a–f** and **6a–c** alone as well as in combination with meropenem (MER).

Sample	MIC/mg dm^{-3}									
	E. coli[a] (TEM-1)	*E. coli*[b] (CTX-M15)	*K. p*[c] (SHV-1)	*K. p*[d] (KPC-3, TEM-1)	*E. c*[e] (P99)	*E. c*[f] (AmpC)	*A. b*[g] (OXA-23/40)	*A. b*[h] (OXA-24)	*P. a*[i] (KPC-2)	*P. a*[j] (AmpC)
3a–f, **6a–c** & avibactam alone	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
MER alone	4	4	4	2	4	4	4	2	4	4
MER + avibactam	<0.125	<0.125	<0.125	<0.125	<0.125	<0.125	1.0	0.5	0.5	0.25
MER + **3a**	1	<0.125	1	<0.125	1	0.5	2	1	1	0.5
MER + **3b**	2	0.5	1	0.5	2	0.5	2	1	2	2
MER + **3c**	2	0.5	1	0.25	4	0.5	2	1	1	0.5
MER + **3d**	1	0.5	1	0.5	1	1	2	0.5	1	0.25
MER + **3e**	1	<0.125	2	0.5	2	2	2	0.5	1	1
MER + **3f**	2	0.25	1	0.5	1	0.25	2	1	2	1
MER + **6a**	<0.125	<0.125	0.25	<0.125	<0.125	<0.125	0.25	0.5	0.5	<0.125
MER + **6b**	<0.125	<0.125	0.25	<0.125	0.25	<0.125	0.25	0.5	1	<0.125
MER + **6c**	0.25	0.25	1	0.25	2	0.5	2	0.5	1	0.5

[a] *E. coli* clinical isolate; [b] *E. coli* 8739; [c] *K. pneumoniae* clinical isolate; [d] *K. pneumoniae* 700603; [e] *E. cloacae* clinical isolate; [f] *E. cloacae* 700323; [g] *A. baumannii* clinical isolate; [h] *A. baumannii* 19606; [i] *P. aeruginosa* clinical isolate; [j] *P. aeruginosa* 9027.

proved to be more resistant to all tested compounds as compared to their wild strains.

In order to compare the efficacy of the synthesized compounds with avibactam, MIC values for MER in combination with avibactam were also determined using same bacterial strains under similar experimental conditions. Comparing the MIC values (see Table 1) for the combination of MER and avibactam with those of MER and compounds **3a**–**f** and **6a**–**c**, it is clear that avibactam is more potent inhibitor than our compounds. However, it can be noted that compounds **6a**,**b** exhibited comparable synergy (MIC, 0.125 to 1 mg dm^{-3}) and showed similar antibacterial profile to avibactam.

A concise relationship between structures of the synthesized compounds and their antibacterial activities would be hard to follow. However, depending on the data obtained for analogous compounds, a few points could be mentioned. For example, *N*-mesylated compounds (**6a**,**b**) showed better activity as compared to their counterpart **6c** with *N*-acyl grouping. Comparing the activity of compounds **3a**–**f** with those of **6a**–**c**, it can be concluded that amide linkage in compounds **6a**–**c** could improve their antibacterial activity.

In conclusion, we successfully synthesized new derivatives of avibactam containing 2-positioned substituted amidine moiety at DBO framework. All compounds did not exhibit antibacterial efficacy when tested alone, however they decreased the MIC of the meropenem in combination. This suggests the β-lactamase inhibition capability of these compounds, as well as it concludes that amide group in avibactam can be replaced by amidine group without loss of its activity. The substituted-amidine analogues of avibactam may be a good choice for future replacement of avibactam.

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Jian Sun and Lili He have equal contribution for this work.

Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2021.07.020.

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