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β -Lactamase inhibition profile of new amidine substituted diazabicyclooctanes

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Abstract: Diazabicyclooctane (DBO) scaffold is the backbone of non-β-lactam based second generation β-lactamase inhibitors. As part of our efforts we have synthesized a series of DBO derivatives **A1-A23** containing amidine substituents at C2 position of the bicyclic ring. These compounds, alone and in combination with meropenem, were tested against ten bacterial strains for their antibacterial activity *in vitro*. All compounds didn't show antibacterial activity when alone (MIC, >64 mg/L), however exhibited moderate inhibition activity in the presence of meropenem by lowering its MIC values. Compound **A12** proved most potent among the other counterparts against all bacterial species with MIC from <0.125 mg/L – 2 mg/L, and is comparable to avibactam against both *E. coli* strains with MIC value of <0.125 mg/L.

Keywords: Amidine, β-lactamases inhibitors, diazabicyclooctane, synthesis, antibacterial activity.

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Introduction

Survival stress posed by the antimicrobial agents triggers multiple mechanisms[1]in microorganisms ultimately leading to the initiation of antibiotic resistance and survival of the microorganisms[2]. In case of Gram-negative pathogenic bacteria, production of β-lactamases[3] is the main arsenal of these microorganisms against antibiotics. The number of β-lactamases is increasing day by day thereby indicating the strength of these pathogens in compromising the efficacy of new antibiotics after certain period of time. Recently WHO warned about the seriousness of carbapenemase resistant Gram-negativebacteria as a global threat and urged for the development of new remedies[4].

β-Lactams (BL) have served as the first line antibiotics since the introduction of penicillin. However, due to existence and continuous increase in β-lactamases[5], multidrug therapy is becoming the new modality of bacterial treatment against multiple-drug resistant (MDR) bacteria. Multidrug therapy employs the combination of an existing antibiotic with a β-lactamase inhibitor (BLI). A few BLI/BL combinations have been approved[6]so far for clinical applications by different countries, clavulanic acid[7]/amoxicillin (Augmentin)[8] being the first one, while others are in clinical trials[6]. Although Augmentin[9] was successfully applied to treat the infections caused by bacterial strains producing Ambler class A and extended spectrum β-lactamases (ESBLs)[10], however the emergence of new and mutant class A β-lactamases compromised its effectiveness overtime[9, 11]. Subsequently sulbactam and tazobactam[12] evolved as the BLI of class A, B and few of class D β-lactamases[13]. These inhibitors were advantageous to clavulanic acid due to their lack of chromosomal induction of AmpC but found susceptible to a few of class A enzymes such as TEM type[9] and CTX-M (ESBL), identified in *Escherichia coli* clinical isolate[13-14].

Diazabicyclooctane (DBO)[15]ring suggested as an alternative to β-lactam ring[16] by the Hoechst researchers[15] could not prove its antibacterial strength in early experiments rather showed β-lactamase inhibition activity. This discovery led the researchers to develop second generation β-lactamase inhibitors, finally succeeded with the approval of avibactam and relebactam as non-β-lactam based BLIs. Avibactam proved potent inhibitor of KPCs, AmpCs and some of class D β-lactamases[17] is now in clinical practice in combination with ceftazidime[6]. Followed by avibactam, relebactam/imipenem/cilastatin[6] combinationhas been approved by FDA for the treatment of clinical indications against carbapenemases, ESBLs, and MDR *Enterobacteriaceae* as well as *Pseudomonas aeruginosa*[18]. Of note these combinations are not effective against class B metallo-lactamases and most of class D (OXA) β-lactamases. Therefore, several other DBO based BLIs[16], such as durlobactam, nacubactam[19], zidebactam, ETX0282 and ARX-1796 (prodrug of Avibactam)[20], WCK 4234[17, 21], are passing through phase I and phase III clinical trials[6, 22] in combination with different types of β-lactams. Of these, WCK 4234 has shown promise against class A, class C and class D carbapenemases[17, 21].

These multidrug combinations have shown promise for future antibiotic regimen and drug development based on non-β-lactam inhibitors. Nonetheless, partial loss of activity has been reported in case of ceftazidime-avibactam combination due to overproduction of AmpC cephalosporinases[23]. In another report it has been concluded that ESBLs of the GES, PER and BEL types in *E. coli* and *P. aeruginosa* conferred resistance against sulbactam and avibactam combinations[24]. Therefore, it is utmost necessary to continue the struggle with exploring new inhibitors capable of improved resistance and activity against all classes of β-lactamases. Based on

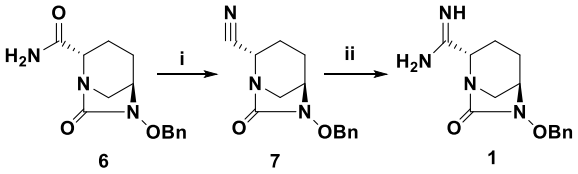
our ongoing efforts towards the synthesis of new DBO based BLIs, we have synthesized a number of amidine conjugated derivatives of avibactam. We report the synthesis and antibacterial as well as inhibitory activities of these compounds in combination with avibactam in comparison to avibactam and meropenem (MER), an existing antibiotic in clinics.

Results and discussion

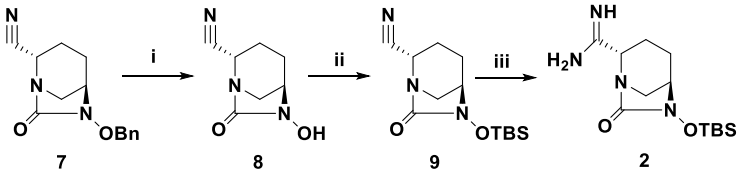
Synthesis of intermediates 1-5

Synthesis of intermdiate **1** is the key step for the synthesis of final compounds (scheme 1). Compound **1** was synthesized by the dehydration of amide[25]**6** which is commercially available. Dehydration was acheived by reacting **6** with trifluoroacetic anhydride in CH_2Cl_2 at room temperature (RT) and is described elsewhere[17]. Conversion of the cyano compound **7** into corresponding amidine compound **1**, the key intermediate, proved cumbersome. Several experiments and reagents were tried before finding the trimethylaluminum ($Al(Me)_3$) and NH_4Cl as the reagents of choice for this conversion. As a result compound **7** was reacted with $Al(Me)_3$ and NH_4Cl to furnish amidine in CH_2Cl_2 starting the reaction at low temperature followed by at ambient temperature for 16 h. Amidine **1** was obtained in 44% yield after purification by columnn chromatography using MeOH and CH_2Cl_2. Lower yield of this reaction was due to the formation of two isomeric products revealed by TLC and subsequent analysis by analytical LCMS. The NMR spectra of both isomers, after chromatographic separation, showed different chemical shifts for the protons at C2 position of DBO ring, indicating the racemization during the reaction process. Less polar isomer with R-configuration at C2 showed complete loss of β-lactamase inhibition activity as compared to the more polar isomer. Therefore less polar isomer was discarded while saving the more polar S-isomer, (relative ratio of S:R isomers = 6:1). Racemization at C2 of DBO

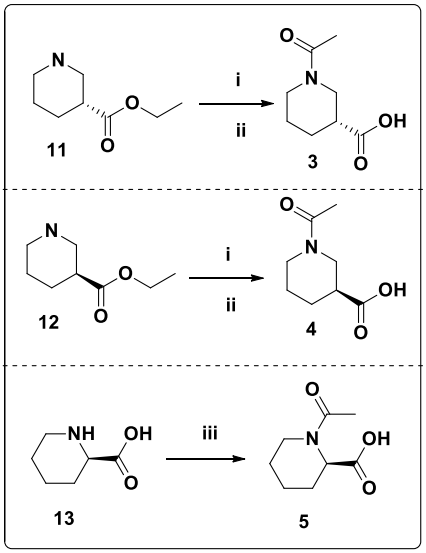
suggests the amidation reaction proceeds through carbocation formation at C2 as well.



Scheme 1. Synthesis of intermediate **1**. *Reagents and condition:* (i) trifluoroacetic anhydride, CH_2Cl_2, 0 °C-RT, 3h; (ii) $Al(Me)_3$, NH_4Cl, CH_2Cl_2, 0 °C-RT, 16h.



Scheme 2. Synthesis of intermediate **2**. *Reagents and conditions:* (i) Pd/C (wet), EtOAc/ CH_2Cl_2, H_2, 45 psi, RT, 2h; (ii) TBSCl, Imidazole, CH_2Cl_2, RT, 16h; (iii) $Al(Me)_3$, NH_4Cl, CH_2Cl_2, 0 °C-RT, 40h.



Scheme 3. Synthesis of intermediates **3-5**. *Reagents and conditions:* (i) $(Ac)_2O$, CH_2Cl_2, RT, 24h; (ii) Aqueous NaOH, 0 °C, 2h; (iii) $(Ac)_2O$, H_2O, RT, 3h.

Synthesis of intermediate **2** started from the hydrogenation of **7** by following previously described method using *N*,*N*-dimethylformamide (DMF)/CH_2Cl_2[17] as solvent led to low yield in our hands.

Therefore, we planned to switch the solvent from DMF to EtOAc whereupon the yield improved however, still amino derivative as side product was observed. Addition of CH_2Cl_2 with ethylacetate proved helpful in increasing the yield and NMR of crude product **8** was acceptable to use it for further reaction without purification. Hydroxyl group in **8** was then protected by TBS (*tert*-butyldimethylsilane) using *tert*-butyldimethylsilyl chloride (TBSCl) and imidazole in CH_2Cl_2. Thus obtained derivative **9** was subjected to amidination by $Al(Me)_3$ and NH_4Cl to afford amidine **2** (scheme 2).

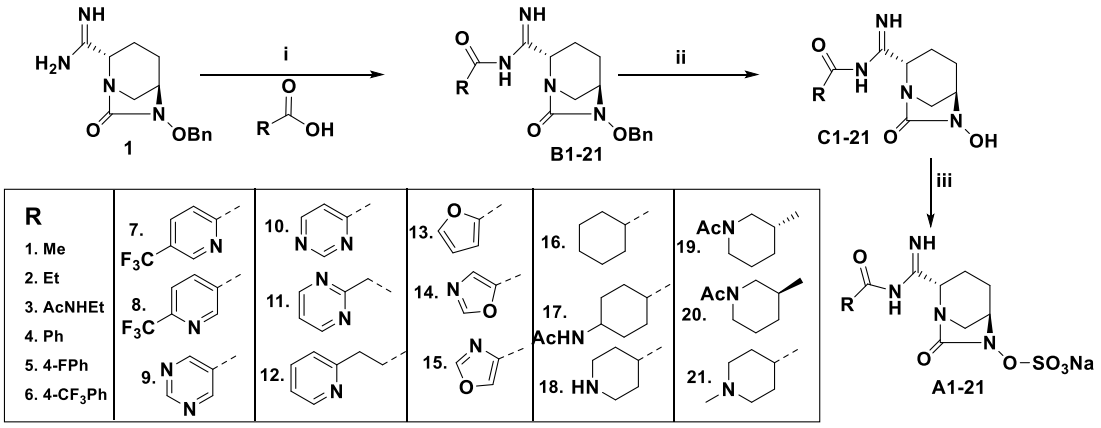
Compounds **3,4** were prepareed from commercially available compounds **11** and **12** respectively in two steps. In first step ester derivatives were acetylated by acetic anhydride in CH_2Cl_2, followed by the hydrolysis by aqueous NaOH in tertrahydrofuran (THF) to afford the required intermediates **3** and **4** in overall good yields. Compound **5** was obtained by direct acetylation of commercially available acid **13** using acetic anhydride and stoicheometric amount of water, at room temeprature (scheme 3).

Synthesis of compounds A1-A23

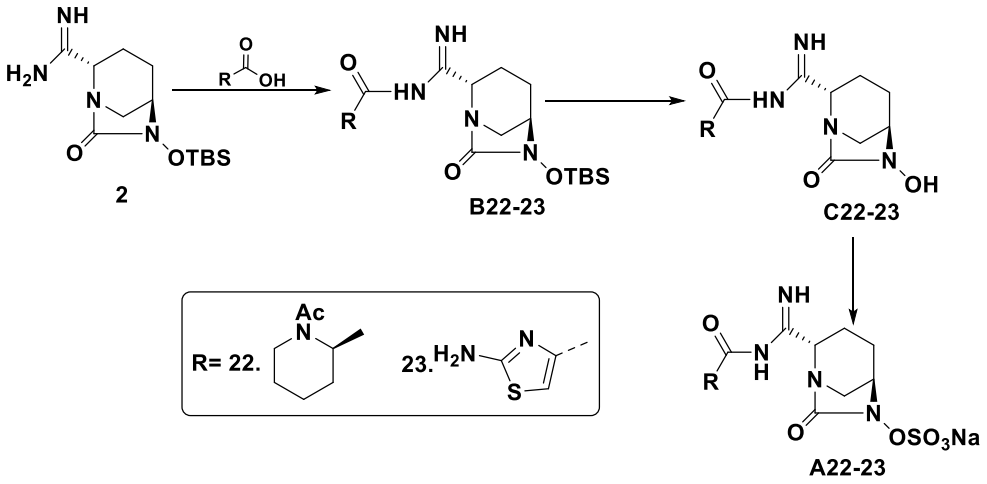
Synthesis of compounds **A1-A21** starting from intermediate **1** was accomplished as depicted in scheme 4. Coupling of the organic acids with amidine **1** to form the corresponding derivatives **B1-B21** was achieved by coupling reagents such as or *N,N'*-dicyclohexylcarbodiimide (DCC) or (O-(7-Aza-1H-benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate) (HATU)[26] in DMF or CH_2Cl_2 whereas *N,N*-diisopropylethylamine (DIPEA) or 4-dimethylaminopyridine (DMAP) were used as base. Palladium catalyzed hydrogenation of compounds **B1-B21** in THF or EtOAc led to afford hydroxy derivatives **C1-C21**. It has been observed that catalytic amount of triethylamine (TEA) in EtOAc enhances the rate of hydrogenolysis of benzyl ethers. Compounds **C1-C21** are then reacted with

SO_3-pyridine to form sulfonic acid derivatives **A1-A21** after purification by preparative HPLC. Sodium salts of these compounds are obtained by ion exchange using column filled with Dowex-50wx Na^+ resin. Water is used as eluant which is lyophilized to get the sodium salts of desired compounds. In case of **A18**, Boc deprotection was applied using trifluoroacetic acid (TFA) before preparative HPLC.

Synthesis of compounds **A22** and **A23** was accomplished by an alternative route elaborated in scheme 5. Coupling of compound **5** with intermediate **2** was done by using HATU and DIPEA in DMF/ CH_2Cl_2 mixture to form the derivative **B22** which was treated with tetrabutylammonium fluoride (TBAF) in THF to obtain the hydroxy derivative **C22**. The compound **C22** was converted to the sodium salt of **A22** by using the procedure described for **A1**. Compound **A23** was prepared following aforementioned scheme 5 methods starting from 4-aminothiazole-2-carboxylic acid and amidine derivative **2** according to the procedures described for **A22**.



Scheme 4. Synthesis of compounds **A1-21**. *Reagents and conditions:* (i) Acetylchloride, TEA, CH_2Cl_2, RT, 16h (for B1); HATU, DIPEA or DCC, DMAP, DMF or THF, RT, 16-24h; (ii) Pd/C (wet), THF or EtOAc/TEA, H_2, RT, 16h; (iii) SO_3-pyridine, pyridine, or SO_3-pyridine, TEA, THF/Water, RT, 16h, then Dowex-50wx Na^+.



Scheme 5: Synthesis of compounds **A22-23.** *Reagents and conditions:* (ii) HATU, DIPEA or DCC, DMAP, DMF or THF, RT, 16h. (iii) TBAF, THF. (iv) SO_3-pyridine, pyridine, or SO_3-pyridine, TEA, THF/Water, RT, 16h, then Dowex-50wx Na^+.

In vitro antibacterial efficacy

We synthesized a series of amidine derivatives of avibactam containing a variety of substituents, forming amide linkage with NH_2 of amidine of the parent intermediate **1** or **2**. Different kinds of substituents (R) introduced in final compounds **A1-A23** are depicted in table 1. *In vitro* antibacterial activities of compounds **A1-A23** were determined without combining it with an antibacterial drug and minimum inhibitor concentration (MIC) of each compound was determined for each of the ten bacterial strains i.e. *E. coli* clinical isolate; *E. coli* 8739; *K. pneumoniae* clinical isolate; *K. pneumoniae* 700603; *E. cloacae* clinical isolate; *E. cloacae* 700323; *A. baumannii* clinical isolate; *A. baumannii* 19606; *P. aeruginosa* clinical isolate and *P. aeruginosa* 9027 (table 1). All the synthesized compounds showed MIC value of >64 mg/L against all tested bacterial species. For comparison, MIC values of avibactam against all of these bacteria were also determined and were found comparable to our synthesized compounds (MIC, >64 mg/L). This indicates that both avibactam and compounds **A1-A23** are not

antibacterial in action when used alone. Next, we determined the antibacterial activity of meropenem (MER) alone and its combination with avibactam as well as in combination with newly synthesized compounds **A1-A23**. From the table 1, it can be deduced that the antibacterial activity of MER increases after addition (4 mg/L) of avibactam against all bacterial strains under observation. The MIC values of MER without avibactam were observed to be in the range of 2 mg/L to 4 mg/L, whereas after the addition of avibactam this range modified to <0.125 mg/L – 1 mg/L indicating the enzyme inhibition effect of the avibactam.

In order to establish the lactamase inhibition effect of our synthesized avibactam derivatives **A1-A23**, we determined the antibacterial activity of MER in combination with compounds **A1-A23** individually. The results are summarised in table 1 as MIC values of each compound against each bactrial strain. From the table it is evident that all of the compounds enhanced the antibacterial activity of MER (MIC, <0.125 mg/L – 2 mg/L) as compared to meropenem alone (MIC, 2 mg/L to 4 mg/L). Compound **A12** proves most potent among the other counterparts against all bacterial species with MIC from<0.125 mg/L – 2 mg/L, and is comparable to avibactam against both *E. coli* strains and *K. pneumoniae* strains with MIC value of <0.125 mg/L. From the data in table 1 it is clear that *A. baumannii* clinical isolate is the most resistant strain against all newly synthesized compounds as well as avibactam showing MIC value of 2 mg/L and 1 mg/L respectively. However, *E. coli* 8739 is the most susceptible strain to most of the synthesized compounds for example, **A1**, **A2**, **A8**, **A12**, **A13** and **A16** with MIC value of <0.125 mg/L.

Table 1. *In vitro* antibacterial activity of avibactam and compounds **A1-A23** alone as well as in combination with meropenem (MER).

R Substituents in **A1-A23**	Sample	Minimum Inhibitory Concentration (MIC, mg/L)									
		E. coli[a]	*E. coli*[b]	*K. p*[c]	*K. p*[d]	*E.c*[e]	*E.c*[f]	*A.b*[g]	*A.b*[h]	*P.a*[i]	*P.a*[j]
	A1-A23& 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	0.5	0.5	0.25
Me	**A1**	0.5	<0.125	2	0.5	2	1	2	0.5	0.5	1
Et	**A2**	0.5	<0.125	2	1	2	2	2	0.5	0.5	0.5
AcNHEt	**A3**	<0.125	0.25	1	0.25	1	1	2	0.5	0.5	1
Ph	**A4**	2	0.25	1	0.25	2	0.25	2	0.5	2	1
4-FPh	**A5**	1	0.25	2	0.25	2	1	2	1	1	1
4-CF_3Ph	**A6**	0.5	0.25	2	0.5	2	2	2	0.5	1	0.5
	**A7**	1	0.25	2	0.5	2	1	2	0.5	0.5	1
	**A8**	0.5	<0.125	2	0.5	2	2	2	0.5	0.5	1
	**A9**	0.5	0.5	2	1	2	2	2	1	1	2
	**A10**	1	0.5	2	0.5	2	1	2	0.5	0.5	1
	**A11**	0.25	0.25	0.25	0.25	0.5	1	2	0.5	0.25	1
	**A12**	<0.125	<0.125	<0.125	<0.125	0.5	0.5	2	1	0.25	0.5
	**A13**	0.5	<0.125	2	0.25	2	1	2	0.5	0.5	1

	**A14**	0.25	0.25	0.25	0.25	2	0.25	2	0.5	0.25	1
	**A15**	0.25	0.25	0.25	0.25	2	1	2	1	0.25	1
	**A16**	1	<0.125	2	0.25	2	0.5	2	0.5	0.5	0.5
	**A17**	0.25	0.25	0.25	0.25	1	1	2	0.5	0.25	0.5
	**A18**	0.25	0.5	0.25	0.25	2	1	2	2	1	0.25
	**A19**	0.25	0.25	2	0.25	1	0.5	2	1	1	0.5
	**A20**	0.25	0.25	1	0.25	2	0.5	2	0.5	1	0.5
	**A21**	0.5	0.5	2	0.5	2	0.5	2	0.5	0.25	1
	**A22**	1	0.5	2	0.25	1	0.5	2	0.5	0.25	0.5
	**A23**	0.25	0.25	1	0.25	0.5	1	2	0.5	0.25	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* 902.

Conclusion

We have successfully synthesized a series of amidine substituted avibactam derivatives in moderate to good overall yields. *In vitro* antibacterial testing for these compounds showed lack of antibacterial efficacy, however all compounds showed moderate lactamase inhibition activity depicted by minimized the MIC values of meropenem in the presence of test compounds. Compound **A12** was most potent inhibitor in case of all bacterial strains under observation and may be a lead compound for further development.

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Graphical Abstract

β-Lactamase inhibition profile of new amidine substituted diazabicyclooctanes

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