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Structure activity relationship of pyridoxazinone substituted RHS analogs of oxabicyclooctane-linked 1,5-naphthyridinyl novel bacterial topoisomerase inhibitors as broad-spectrum antibacterial agents (Part-6)

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ABSTRACT

Oxabicyclooctane linked 1,5-naphthyridinyl-pyridoxazinones are novel broad-spectrum bacterial topoisomerase inhibitors (NBTIs) targeting bacterial DNA gyrase and topoisomerase IV at a site different than quinolones. Due to lack of cross-resistance to known antibiotics they present excellent opportunity to combat drug-resistant bacteria. A structure activity relationship of the pyridoxazinone moiety is described in this Letter. Chemical synthesis and activities of NBTIs with substitutions at C-3, C-4 and C-7 of the pyridoxazinone moiety with halogens, alkyl groups and methoxy group has been described. In addition, substitutions of the linker NH proton and its transformation into amide analogs of AM-8085 and AM-8191 have been reported. Fluoro, chloro, and methyl groups at C-3 of the pyridoxazinone moiety retained the potency and spectrum. In addition, a C-3 fluoro analog showed 4-fold better oral efficacy (ED_{50} 3.9 mg/kg) as compared to the parent AM-8085 in a murine bacteremia model of infection of *Staphylococcus aureus*. Even modest polarity (e.g., methoxy) is not tolerated at C-3 of the pyridoxazinone unit. The basicity and NH group of the linker is important for the activity when CH_2 is at the linker position-8. However, amides (with linker position-8 ketone) with a position-7 NH or *N*-methyl group retained potency and spectrum suggesting that neither basicity nor hydrogen-donor properties of the linker amide NH is essential for the activity. This would suggest likely an altered binding mode of the linker position-7,8 amide containing compounds. The amides showed highly improved hERG (functional IC_{50} >30 μM) profile.

Drug resistance bacteria continue to rise and pace of the discovery of new antibacterial agents to combat such bacteria continue to recede. Recently described bacterial type II topoisomerase inhibitors (NBTIs) are the newest classes of antibacterial agents that show broad-spectrum of activity without cross-resistance to known classes of antibiotics and thus has potential to address unmet need.[1–11] The lack of cross-resistance is inherent to their binding mode to DNA gyrase and topoisomerase IV at a site different from fluoroquinolones (e.g., ciprofloxacin **1**, Fig. 1) and other known antibiotics. GSK2140944 (**2**)[12] is under phase II human clinical development for AbSSTI and Gonorrheal diseases (clinicaltrials.gov) and is the most advanced of the NBTIs. We have been studying NBTI class of DNA gyrase inhibitors and recently reported several oxabicyclooctane linked 1,5-naphthyridinyl-pyridoxazone NBTIs including AM-8085 (**3**) and AM-8191 (**4**)[13] as well as several hydroxy tricyclic series of NBTIs.[14] Development of NBTIs in general and this series in particular has been hampered due to existence of significant hERG K^+ channel activity.

AM-8085 and AM-8191 series of oxabicyclooctane linked NBTIs show broad-spectrum activity covering Gram-positive and Gram-

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0960-894X/

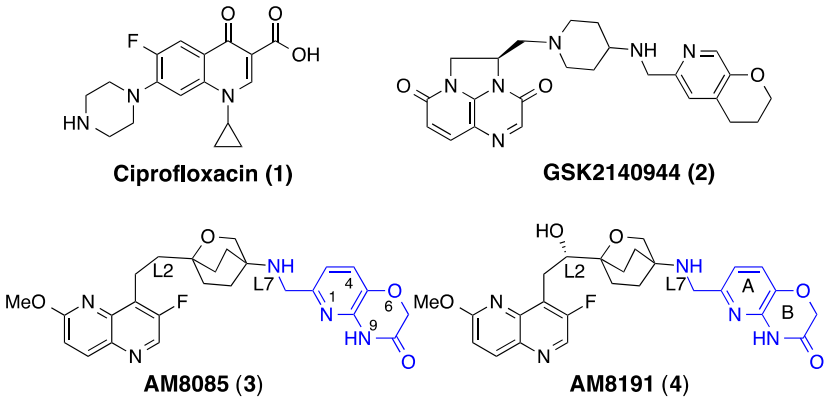


Figure 1. Chemical structures of NBTIs.

negative pathogens and in vivo efficacy with modest attenuation of hERG signal.[13] The hydroxy tricyclic series of NBTIs showed significant attenuation of hERG signal unfortunately at the expense of potency and spectrum against Gram-negative pathogens.[14] In order to maintain Gram-negative activity and spectrum, we focused our SAR study on the both series. Recently we reported SAR around the LHS 1,5-naphthyridine[15,16] and hydroxy tricyclic 1,5-naphthyridinone,[14] as well as a survey of RHS moieties applicable to the hydroxy tricyclic series.[17] This Letter focuses on the synthesis and SAR of pyridoxazinone-substituted oxabicyclooctane linked NBTIs along with the defining an understanding of the role of the NH at position-7 of the linker.

Chemistry. The chemical synthesis of right-hand-side (RHS) pyridoxazinone substituted NBTIs (**5**–**25**, Tables 1–3) are described in Schemes 1–9. Chlorination of the intermediate **26**[13] gave **27** which was transformed to E-styrene **28** by Suzuki coupling and was oxidized to produce **29**. Standard reductive amination of with **30a**[13] afforded the chloro NBTI **5** (Scheme 1). Fluorination of **31** gave 3,5-difluoro pyridine **32**, which was acylated to give *N*-acyl derivative **33** (Scheme 2). Refluxing of **33** under base catalysis produced the cyclic acid **35** (via methyl ester **34**), which was reduced to alcohol **36** and oxidized with MnO_2 to afford fluoro-aldehyde **37**. The aldehyde **37** was reductively aminated with amines **30a** and **30b**[13] to furnish final fluoro NBTIs **6** and **23**, respectively (Scheme 2).

The nitration of 3-bromo-5-hydroxy-pyridine (**38**) produced 6-nitro-pyridine (**39**) (Scheme 3). Halogen displacement with methoxide afforded methoxy pyridine (**40**). Bromination gave **41** and alkylation produced **42** followed by reduction and cyclization gave **43**. Standard protocol using Suzuki coupling (**44**), oxidation (**45**) and reductive amination with appropriate amines (**30a**, **30b**) afforded NBTIs **7** and **24** (Scheme 3). The 3-methyl substituted NBTI **8** was synthesized from the aldehyde **46**[13] via reduction (**47**), bromination (**48**), Suzuki coupling (**49**) and oxidation (**50**) followed by reductive amination with **30a** (Scheme 4). 4-Methyl NBTIs **9**, **10**, and **14** were prepared starting from substituted pyridine (**51**) via N-oxidation (**52**) followed by acetylation (**53**), hydrolysis (**54**), nitration (**55**), bromination (**56**) and standard stepwise operations (**57**–**60**) as described in Scheme 5.

The reduction of aldehyde **61** (prepared in Scheme 5) gave alcohol **62** which was brominated (**63**) followed by Suzuki coupling gave vinyl analog **64**. Hydrogenation of **64** to **65** and oxidation to **66** followed by reductive amination with **30a** gave 3-ethyl-4-methyl NBTI **11** (Scheme 6). Oxidation of **64** gave **67** followed by reductive amination produced 4-vinyl-4-methyl NBTI **13** (Scheme 6). Suzuki coupling of **63** gave 3-isopropenyl-4-methyl pyridine (**68**), hydrogenated to afford (**69**), oxidized to give aldehyde **70** and reductive amination afforded isopropyl NBTI **12** (Scheme 7).

Alkylation of 2-bromo-5-hydroxy-6-amino-pyridine (**71**) followed by reflux produced substituted pyridoxazinone **72**. Suzuki coupling gave **73** and ozonolysis yielded produced **74**, which upon reductive amination afforded the geminal dimethyl NBTI (**16**) and a racemic NBTI **17**. The chiral HPLC separation of the racemic **17** furnished both enantiomers (+)-**17** and (−)-**17** (Scheme 8). Hydrolysis, decarboxylation and ozonolysis of **75** produced aldehyde **76** which was reductively aminated with **30a** to afford racemic **15** which was separated by chiral HPLC to give enantiomers (+)-**15** and (−)-**15** (Scheme 8). Methylation of the amide nitrogen of **77** gave **78**, which under standard operations gave NBTI **18**. The amide NBTIs **21**, **22**, **25** were prepared by EDC couplings of the acid **79**[17] with amine **30a**, *N*-methyl-**30a** and **30b**, respectively (Scheme 9). The linker at position L7 substituted NBTIs **19** and **20** were synthesized by reductive methylation with paraformaldehyde and reaction with chloroformate, respectively. All final products were well characterized by NMR and MS. The purity of all compounds was greater than 95% as assessed by ^{1}H NMR and HPLC.

The X-ray crystal structure of AM-8191 bound to DNA gyrase-DNA complex (GyrB27-A56 protein construct and 12–37 bp DNA duplex, pdb code; 4plb) showed that the 1,5-naphthyridine ring was sandwiched between two base pairs of the DNA at the top and RHS moiety pyridoxazinone penetrated into the hydrophobic pocket created by the two Gyrase A subunits at the bottom portion of the complex.[13] The pyridoxazinone binding was stabilized by van der Walls interactions of the pyridine ring with Ala68, Val71, Met75 and Met121. While specific structure-guided modifications have been not successful in this program nevertheless it was envisaged that introduction of various hydrophobic groups on pyridoxazinone could provide NBTIs with gain of function likely from additional hydrophobic interactions. Understanding SAR, balancing potency, spectrum and hERG activity was the goal of this study. Carefully selected small set of hydrophobic groups was used for substitution of the hydrogen at the C-3 of AM-8085/AM-8191 to give chloro (**5**), fluoro (**6**, **23**), and methyl (**8**) substituted NBTIs. C-3 methoxy compounds (**7**, **24**) were prepared to confirm the minimum tolerance of polarities. With a similar rationale, C-4 methyl (**9**) and C-3,4-disubstituted analogs with small changes in the size and shapes of alkyl groups (**10**–**13**) at C-3 as well as a C-3 fluoro group (**14**) while maintaining a methyl at C-4 were prepared. Likewise to understand substitutions at C-7, enantiomers of mono-methyl analogs (**15**), a geminal dimethyl analog (**16**) were prepared. In addition, to test whether a polar group was tolerated at C-7 of pyridoxazinone, both enantiomers of the 7-methyl-7-ethoxy carbonyl (**17**) were also prepared. The NH group of the pyridoxazinone was methylated (**18**) to test the role of the NH group. The X-ray crystal structure suggested that the basic nitrogen at

Table 1
Antibacterial activity and spectrum pyridoxazinone substituted AM-8085 oxabicyclooctane linked NBTIs (MIC, μg/mL)[a]

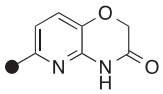
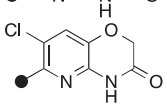
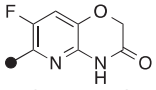
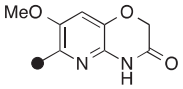
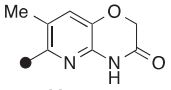
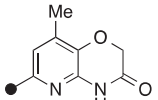
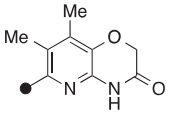
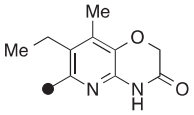
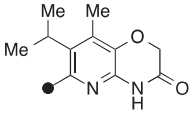
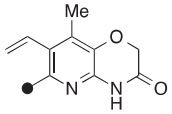
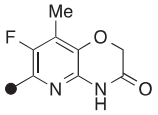
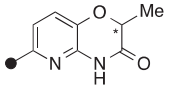
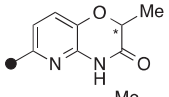
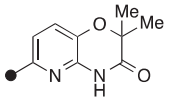
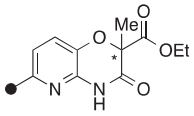
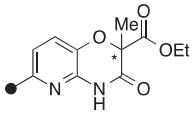
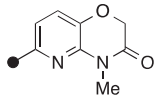
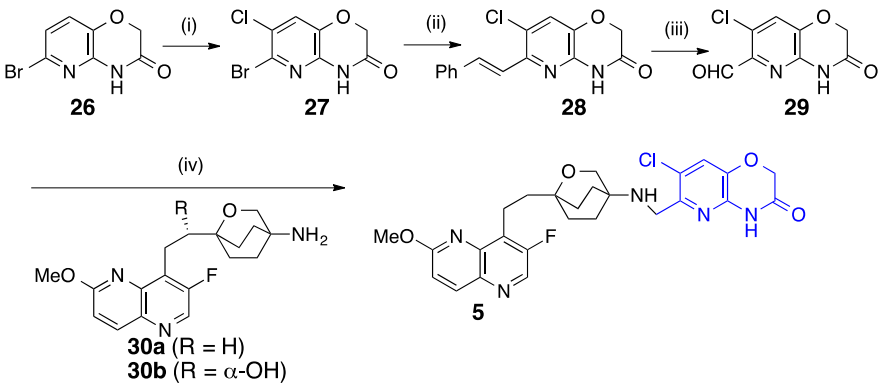
List	RHS	SaS	Sp	Ef	Ec	Ab	Pa	hERG bind /PX hERG (IC_{50}, μM)	$c\log D_{7.4}$
3		0.02	0.13	0.5	1	0.5	4	2.0/0.6	3.2
5		0.02	0.25	1	2	1	2	5.8	3.98
6		0.02	0.13	1	2	0.5	8	1.3	3.41
7		4	8	8	8	8	8		3.58
8		0.06	1	1	2	0.5	8	1.0/4.3	3.56
9		0.50	4.0	8.0	8	4	32	0.3/2.0	3.61
10		1.06	8.0	2	2	4.0	16	0.5/4.6	4
11		2	32	32	32	32	32	0.7	4.53
12		8	32	32	32	32	32	1.2	4.95
13		0.25	8	32	32	32	32	0.5	4.37
14		0.5	4	32	32	32	32	0.9	3.91
(+)-**15**		0.25	0.25	2	2	2	2	0.8	3.61
(−)-**15**		0.06	0.25	2	2	1	2	1.8	3.61
16		0.25	1	2	2	2	2	3.0	4.13
(+)-**17**		16	16	16	16	16	16	2.4	3.96
(−)-**17**		8	8	8	8	8	8	3.6	3.96

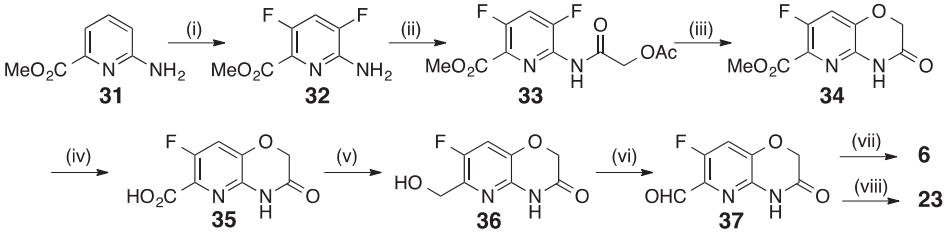
Table 1 (*continued*)

List	RHS	SaS	Sp	Ef	Ec	Ab	Pa	hERG bind /PX hERG (IC_{50}, μM)	$clogD_{7.4}$
18		2	2	16	16	16	16	0.8	2.59

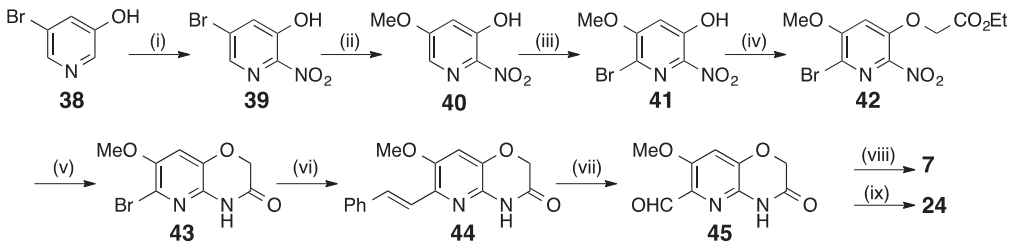
[a] SaS (*Staphylococcus aureus* Smith, MIC against MRSA OIT971 similar), Sp (*Streptococcus pneumoniae* IID553), Ef (*Enterococcus faecium* VanA, VRE), Ec (*Escherichia coli* ATCC 25922), Ab (*Acinetobacter baumannii* IID876), Pa (*Pseudomonas aeruginosa* PAO1), hERG binding (MK499 binding), PX hERG (Patch express, CHO cell),[13] Linezolid and levofloxacin were used as controls for the MIC measurements using the micro broth dilution or agar based methods which yielded the reported the MIC ranges reported by the Clinical and Laboratory Standards Institute (CLSI M7-A8). AM8085 showed a 4- and 2-fold increase in the MIC under the same test conditions, respectively. All compounds experienced less than 4 fold MIC shifts against quinolone sensitive and resistant strains of *S. aureus* MS5935 $quin^S$ vs MS5935 $quin^R$). For comparison, the MIC values of levofloxacin was >64 fold higher with the quinolone resistant (*S. aureus* MS5935 $quin^R$) as compared to the sensitive (*S. aureus* MS5935 $quin^S$) strain, (>16 vs 0.25 μg/mL), NT (not tested). The data for compounds **3** is taken from Singh et al.[13]



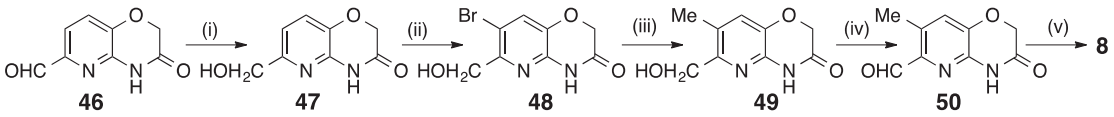
Scheme 1. Synthesis of NBTI **5**. Reagents and conditions: (i) Cl_2, DMF, (ii) K_2CO_3, 1,4-dioxane–water, phenylvinylboronic acid, $Pd(PPh_3)_4$, (iii) O_3, CH_2Cl_2, MeOH, −71 °C, DMS, (iv) **30a**, DMF, AcOH, $NaBH(OAc)_3$, rt.



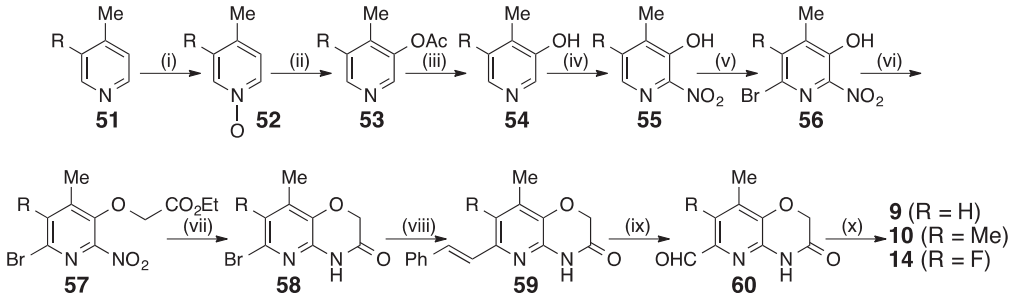
Scheme 2. Synthesis of NBTI **6** and **23**. Reagents and conditions: (i) F–TEDA–BF_4, CH_3CN, (ii) $ClCOCH_2OAc$, pyridine, (iii) K_2CO_3, MeOH, reflux, (iv) NaOH, 1,4-dioxane–water, (v) (a) *i*-butyl chloroformate, Et_3N, DMF, (b) $NaBH_4$, H_2O, (vi) MnO_2, THF, (vii) **30a**, DMF, AcOH, $NaBH(OAc)_3$, rt, (viii) **30b**, DMF, AcOH, $NaBH(OAc)_3$, rt.



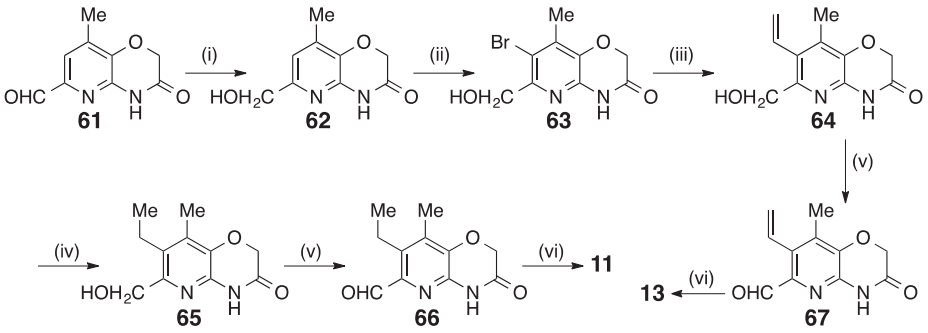
Scheme 3. Synthesis of NBTI **7** and **24**. Reagents and conditions: (i) HNO_3, H_2SO_4, (ii) NaOMe, Cu, CH_3OH, microwave, 150 °C, (iii) benzyltrimethylammonium tribromide, CH_2Cl_2, (iv) K_2CO_3, acetone, $BrCH_2CO_2Et$, reflux, (v) Fe, AcOH, 90 °C, (vi) K_2CO_3, 1,4-dioxane–water, phenylvinylboronic acid, $Pd(PPh_3)_4$, (vii) O_3, CH_2Cl_2, MeOH, −71 °C, DMS, (viii) **30a**, DMF, AcOH, $NaBH(OAc)_3$, rt, (ix) **30b**, DMF, AcOH, $NaBH(OAc)_3$, rt.



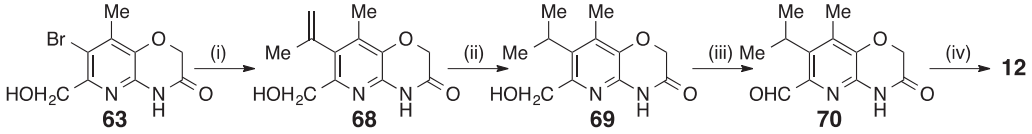
Scheme 4. Synthesis of NBTI **8**. Reagents and conditions: (i) $NaBH_4$, MeOH, (ii) Br_2, DMF, (iii) $MeB(OH)_2$, 1,4-dioxane, $Pd(dppf)_2Cl_2$, K_2CO_3, (iv) MnO_2, THF–CH_2Cl_2 (1:1), (v) **30a**, DMF, AcOH, $NaBH(OAc)_3$, rt.



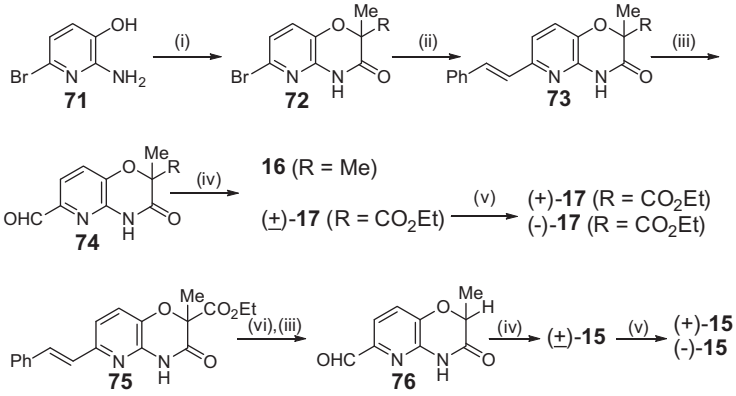
Scheme 5. Synthesis of NBTI **9**, **10**, **14**. Reagents and conditions: (i) H_2O_2, AcOH, reflux, (ii) Ac_2O, reflux, (iii) NaOH, EtOH, (iv) HNO_3, H_2SO_4, (v) NaOMe, MeOH, Br_2, ice bath, (vi) K_2CO_3, acetone, $BrCH_2CO_2Et$, reflux, (vii) Fe, $CaCl_2$, EtOH, reflux, (viii) K_2CO_3, 1,4-dioxane, phenylvinylboronic acid, $Pd(PPh_3)_4$, (ix) O_3, CH_2Cl_2, MeOH, −71 °C, DMS, (xi) **30a**, DMF, AcOH, $NaBH(OAc)_3$, rt.



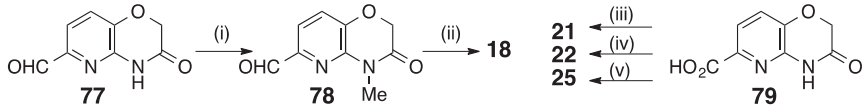
Scheme 6. Synthesis of NBTI **11**, **13**. Reagents and conditions: (i) $NaBH_4$, MeOH, (ii) Br_2, DMF, ice bath, (iii) $CH_2{=}CHBF_3K$, EtOH, Et_3N, reflux, (iv) Pd/C, MeOH, H_2, (v) MnO_2, THF–CH_2Cl_2 (1:1), reflux, (vi) **30a**, DMF, AcOH, $NaBH(OAc)_3$, rt.



Scheme 7. Synthesis of NBTI **12**. Reagents and conditions: (i) 2-isopropenyl-4,4,5,5-tetramethyl-[1,3,2]dioxaborolane, $Pd(dppf)_2Cl_2$, Na_2CO_3, 1,4-dioxane, water, (ii) Pd/C, MeOH, H_2, (iii) MnO_2, THF–CH_2Cl_2 (1:1), reflux, (iv) **30a**, DMF, AcOH, $NaBH(OAc)_3$, rt.

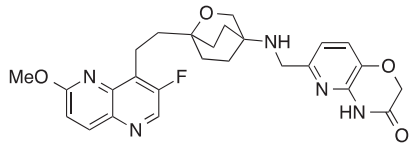
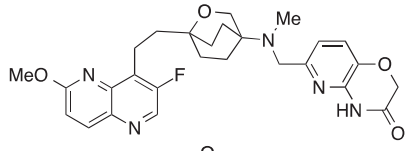
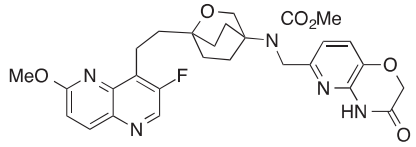
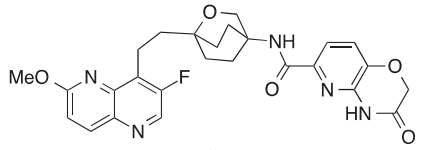
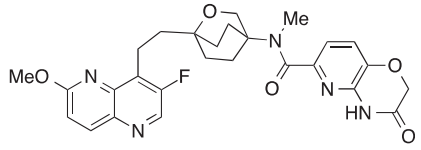


Scheme 8. Synthesis of NBTI **15**–**17**. Reagents and conditions: (i) Br–C(Me,R)CO_2Et, K_2CO_3, acetone, reflux, (ii) K_2CO_3, 1,4-dioxane–water, phenylvinylboronic acid, $Pd(PPh_3)_4$, (iii) O_3, CH_2Cl_2, MeOH, −71 °C, DMS, (iv) **30a**, DMF, AcOH, $NaBH(OAc)_3$, rt, (v) Chiralpak IA, hexane–EtOH (1:4) +0.1% Et_2NH, (vi) K_2CO_3, 1,4-dioxane–water, reflux.



Scheme 9. Synthesis of NBTI **18**, **21**, **22**, **25**. Reagents and conditions: (i) MeI, benzyltriethylammonium chloride, K_2CO_3, CH_3CN, (ii) **30a**, DMF, AcOH, $NaBH(OAc)_3$, rt, (iii) **30a**, EDC, HOBT, DIEA, CH_2Cl_2, (iv) N–Me–**30a**, EDC, HOBT, DIEA, CH_2Cl_2, (v) **30b**, EDC, HOBT, DIEA, CH_2Cl_2.

Table 2
Antibacterial activity and spectrum of linker (L7) and amide substituted AM-8085 oxabicyclooctane linked NBTIs (MIC, μg/mL)[a]

List	RHS	SaS	Sp	Ef	Ec	Ab	Pa	hERG bind/PX hERG (IC_{50}, uM)	$c\log D_{7.4}$
3		0.02	0.13	0.5	1	0.5	4	2.1/0.6	3.2
19		0.13	2	8	16	8	32	1.2	3.67
20		16	64	64	64	64	64	6.1	3.92
21		0.02	0.5	1	8	8	8	>60.0/>30.0	3.67
22		0.03	4	4	4	4	4	>60.0/>30.0	2.97

[a] See footnote of Table 1. The data for compound **3** is taken from Singh et al.[13]

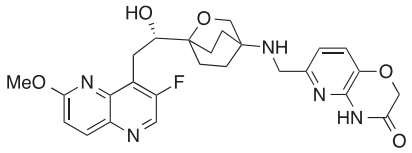
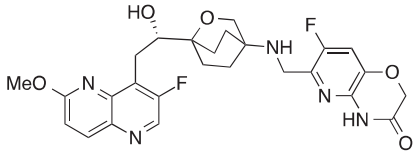
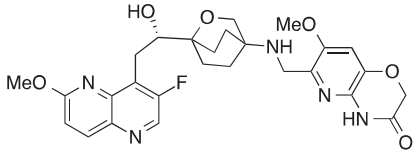
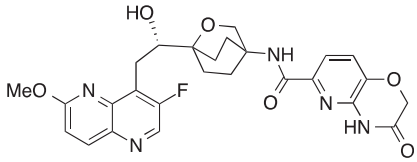
position-7 (L7) of the linker showed a high affinity polar interaction with the Asp83 side chain. In order to test the role of this interaction for potency and spectrum we prepared several N-alkylated analogs (**19** and **20**) and amide analogs (**21**, **22**, **25**).

NBTIs **5**–**25** were tested as a freebase or hydrochloride salt(s) for their potency (MIC) and spectrum against selected Gram-positive and Gram-negative bacterial strains (Tables 1–3). The activities of **3** and **4** have been listed for comparison. In our hands, freebase and the corresponding hydrochloride salt did not show in vitro MIC activity difference. For in vivo activity, only hydrochloride salts were used. The Gram-positive strains included *Staphylococcus aureus*, methicillin resistant *S. aureus*, *Streptococcus pneumoniae*, *Enterococcus faecium*, and Gram-negative strains *Escherichia coli*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*. Since no MIC difference between *S. aureus* Smith and MRSA strains was observed only *S. aureus* Smith MIC data has been reported in Tables 1–3.

The C-3 chloro (**5**) and fluoro (**6**) pyridoxazinone analogs showed similar potency and spectrum as AM-8085 (Table 1) unfortunately without significantly impacting hERG signal. The methyl substitution at C-3 (**8**) had marginal impact on the activity with the exception of *S. pneumoniae*, which showed 8-fold reduction of the MIC. On the other hand, a C-4 methyl (**9**) substitution had negative effect on the potency and spectrum (Table 1). Even a modestly polar group such as a methoxy group (**7**) was not tolerated at C-3. These observations were consistent with the observed results of C-3 pyridoxazinone substituted hydroxy tricyclic series.[17] A methyl substitution at C-4 with a methyl at C-3 (**10**) lost some potency but maintained susceptibility against most strains except *S. pneumoniae* (MIC 8 μg/mL) and *P. aeruginosa* (MIC 16 μg/mL). NBTIs with a slightly larger alkyl groups (e.g., ethyl, **11**, and vinyl, **13**) and a fluoro group (**14**) at C-3 along with a methyl at C-4 showed good *S. aureus* activity (MIC 0.5–2 μg/mL), modest to weak *S. pneumoniae* activity (MIC 4–32 μg/mL), and highly diminished activity (MIC 32 μg/mL) against other strains (Table 1). The bulky isopropyl group (**12**) showed no activity (MIC 32 μg/mL). NBTIs with mono (**15**) and dimethyl (**16**) substitutions at C-7 of pyridoxazinone exhibited reduction of the potency against *S. aureus* but showed flat balanced potency against all other strains and maintained the spectrum. No significant stereochemical preference was observed. NBTIs with C-7 ethyl carboxylate (**17**) substitution showed significant diminution of the activity regardless of the stereochemistry. NH of pyridoxazinone (**18**) appears to be critical for the potent antibacterial activities (Table 1).

The substitution of the hydrogen of basic NH at the position-7 of the linker (L7) with a methyl group (**19**) reduced activity against all strains by 6–16 folds (Table 2). On the other hand substitution with a carboxy methyl group (**20**) significantly diminished activity (MIC 16–32 μg/mL). The amide linked compounds with or without NH (**21** and **22**) retained potent *S. aureus* activity (MIC 0.02–0.03 μg/mL) while maintaining spectrum with a 2–4 folds loss of the potency against other strains. These data suggest that a hydrogen-bond donor basic NH appears to be important for potent *S. aureus* cellular activity. However, when the position-7 nitrogen was attached to a keto group (linker position-8) forming an amide, the requirement of the hydrogen-bond donor group for the cellular activity was not essential particularly for *S. aureus* activity. Both NH and *N*-methyl amides and the parent **3** exhibited identical potency against *S. aureus* and maintained spectrum. This would suggest that basicity of the nitrogen or free NH at position-7 of the linker is not essential for the cellular activity in *S. aureus* for the amides not only in AM8085 series but also tricyclic series.[17] Whether this is due to altered target binding or altered cellular accumulation of **21** and **22** need further exploration. The amides (**21** and **22**) showed significant attenuation of the hERG binding (IC_{50} >60 μM) and functional (IC_{50} >30 μM) activities.

Table 3
Antibacterial activity and spectrum of pyridoxazinone substituted AM-8191 oxabicyclooctane linked NBTIs (MIC, μg/mL)[a]

List	RHS	SaS	Sp	Ef	Ec	Ab	Pa	hERG bind/PX hERG (IC_{50}, μM)	$clogD_{7.4}$
4		0.02	0.05	0.5	2	0.5	8	26.0/18.0	1.9
23		0.03	0.25	2	4	1	16	2.4	2.13
24		16	16	16	16	16	16	NT	2.31
25		0.03	2	2	16	2	32	NT	2.4

[a] See footnote of Table 1. The data for compound **4** was taken from Singh et al.[13]

Table 4
In vivo activity of NBTI's in *S. aureus* murine survival model of bacteremia by intravenous (iv) and oral (po) dosing[a]

List	iv (ED_{50}, mpk)	po (ED_{50}, mpk)
3	5.5	17
4	2.5	2.2
6	2.5	3.9
(−)-**15**	14	28
23	3.2	6.7

[a] Dosing vehicle: iv [(2 mg/mL in EtOH/PEG400/water (10:25:65), clear solution], po [(tween 80:0.5% methocel (10:90)].

The C-3 fluoro (**23**) and C-3 methoxy (**24**) and the amide **25** of AM-8191 series showed similar effect on the corresponding MIC values as observed for AM-8085 series and thus confirmed cross-series SAR (Table 3).

The hydrochloride salts of **6**, (−)-**15**, and **23** representing C-3 fluoro analogs of AM-8085 and AM-8191 and the C-7 methyl analog of AM-8085 with potent *S. aureus* MIC (0.02–0.06 μg/mL) were tested in the murine bacteremia survival model of *S. aureus* infection.[13] Their efficacy was compared with the reported efficacy of AM-8085 and AM-8191 (Table 4). 50 mg/kg was used as the highest starting dose for all compounds. The fluoro analog (**6**) of AM-8085 showed about two-fold (ED_{50} 2.5 mg/kg) improved intravenous efficacy and over four-fold (ED_{50} 3.9 mg/kg) improved oral efficacy compared to AM-8085 likely due to improved metabolic stability. The efficacy of the hydroxy fluoro analog **23** was somewhat inferior to the parent AM-8191 (Table 4). The efficacy of the C-7 methyl analog (−)-**15** was significantly decreased. The PK data of these compounds was not collected.

In summary, this Letter describes the SAR of C-3, C-4 and C-7 substituted pyridoxazinone oxabicyclooctane linked 1,5-naphthyridinyl NBTIs. The study concludes that small hydrophobic groups are tolerated at C-3 but not at C-4 or C-3 and C-4. A C-3 Fluoro analog (**6**) showed four-fold improvement of in vivo oral efficacy. Methyl substitution at C-7 showed slightly diminished but flat potency and spectrum including activity against *P. aeruginosa* and would benefit from additional studies. In general basicity and hydrogen donating properties of NH of the linker at position-7 is important for the potency and spectrum but compounds in which NH is tied in the form of an amide bond neither basicity nor NH is critical for the potency and spectrum. Importantly, amides showed significant improvement of the functional hERG activity.

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