Discovery of a teraryl oxazolidinone compound (*S*)-*N*-((3-(3-fluoro-4-(4-(pyridin-2-yl)-1*H*-pyrazol-1-yl)phenyl)-2-oxooxazolidin-5-yl)methyl) acetamide phosphate as a novel antimicrobial agent with enhanced safety profile and efficacies

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Abstract

A series of novel teraryl oxazolidinone compounds was designed, synthesized and evaluated for their antimicrobial activity and toxicities. The compounds with aromatic *N*-heterocyclic substituents at the 4-position of pyrazolyl ring showed better antibacterial activity against the tested bacteria than other compounds with different pattern of substitution. Among all potent compounds, **10f** exhibited promising safety profile in MTT assays and in hERG K^+ channel inhibition test. Furthermore, its phosphate was found to be highly soluble in water (47.1 mg/mL), which is beneficial for the subsequent *in vivo* test. In MRSA systemic infection mice models, **10f** phosphate exerted significantly improved survival protection compared with linezolid. The compound also demonstrated high oral bioavailability (F = 99.1%). Moreover, from the results of *in vivo* toxicology experiments, **10f** phosphate would be predicted to have less bone marrow suppression.

Keywords: oxazolidinones; antibacterial agents; potency; solubility; toxicity.

INTRODUCTION:

The development of bacterial resistance to currently available antibiotics is a growing global health threat[1–3]. Infectious diseases caused by resistant Gram-positive bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA), penicillin-resistant *Streptococcus pneumonia* (PRSP), and vancomycin-resistant *Enterococcus faecalis* (VRE) are of particular concern[4–6]. Linezolid (**1**) (Figure 1), the first marketed and unique antibacterial agent of oxazolidinone family, has been proven to bind with the 50S ribosomal subunit to inhibit bacterial translation at the initiation phase of protein synthesis. This agent demonstrates good activity against all major pathogenic Gram-positive bacteria, including MRSA, vancomycin-resistant *Enterococcus faecium* (VREF) and penicillin-resistant *Streptococcus pneumonia*[7]. The success of linezolid stimulated considerable efforts to search for improved agents in the oxazolidinone class. Several oxazolidinone drug candidates are currently undergoing clinical investigation at different hospitals worldwide[8–9]. As stated above, linezolid acts by the inhibition of protein synthesis through binding to 50S subunit of bacterial ribosome, with a similar mechanism ascribed to the inhibition of mitochondrial protein synthesis in mammals. This mode of action is fundamentally associated with its biochemical mechanisms responsible for the main adverse effect of myelosuppression[10–11]. To our knowledge, several novel oxazolidione antibacterial research and development programs were discontinued because of safety issues. In consequence, improving the balance between efficacy and toxicities in a campaign on discovery and translational research of novel oxazolidione drug candidates is crucial.

Adam and his co-workers[12] have recently performed structural modification of linezolid in C-ring part, attenuating mitochondrial protein synthesis inhibitory side effects, while retaining the desired antibacterial effect. As such, for the best combination of potency and host tolerability, we focus our

efforts on the modification of the C-ring of linezolid guided by results of docking experiments.

Previously reported modeling studies have demonstrated that oxazolidinone compounds with various C-ring attachments could fit nicely with the 50S ribosomal subunit[13]. For instance, compounds **3** and **4** (Figure 1) could bind well to the 50S subunit through hydrogen bond or π-π interaction. The induced-fit mechanism allows the ribosome pocket to adopt a distinct position to accommodate different chemical spaces of oxazolidinone compounds[14]. This *in silico* hypothesis prompts us to explore the diverse chemical space of C-ring of linezolid.

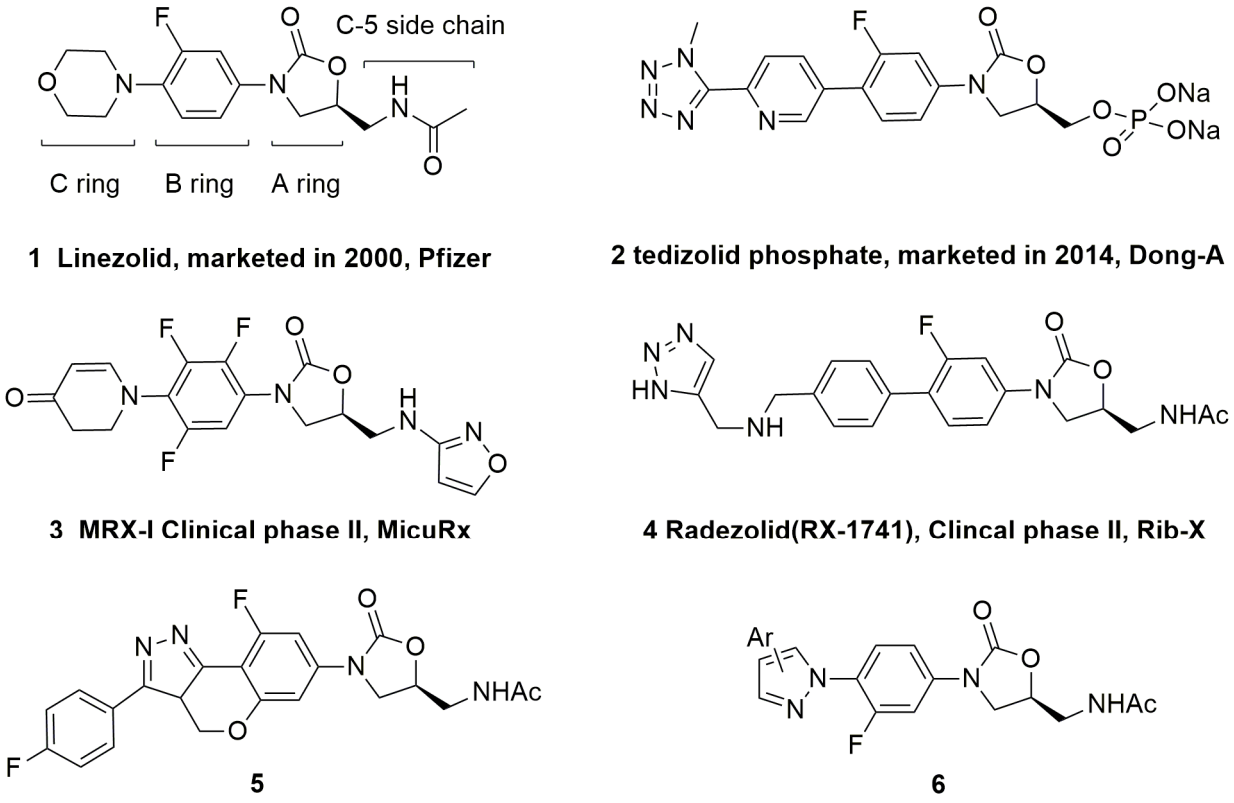


Figure 1. Chemical structures of linezolid and other oxazolidinones

As indicated by previous studies[8, 13], biaryl- or teraryl-featuring oxazolidinones, like compounds **2**, **4**, and **5** (Figure 1), are becoming increasingly known for their high potential as novel antimicrobial candidates. Compound **2**, which contains a biaryl substituent at the C ring position of linezolid and performs an essential function in drug–receptor interaction, has attracted considerable interest. However,

its teraryl moiety appears to be relative rigid and linear like, and then, this kind of oxazolidinones would be a small fraction of all teraryl moiety-featuring oxazolidinone compounds. Elucidating the potential diversity of the substituents in the linezolid C-ring is highly beneficial. The linear conformation of **2** likely enables it to bind well with the pocket and thus exhibit excellent activity. This interesting feature motivated us to determine whether compounds without a straight-line conformation also had good activity. Thus novel oxazolidinone compounds featuring multi-directional biaryl C rings were designed and synthesized with the purpose of discovering oxazolidinone antibacterial agents with enhanced potency and attenuated toxicology. Herein, an aromatic-substituted pyrazolyl ring was used as our biaryl substructure, in which a pyrazolyl ring was retained as the common feature in all target molecules because of its high efficiency in generating biaryl moieties with multi-directional projections (Structure **6**, Figure 1). The resultant biaryl C ring connects fluorophenyl B ring through C-N bond, while various aromatic rings could be introduced at 3-, 4- and 5- positions of the pyrazolyl ring. The aromatic ring at 4- position could be achieved through the reaction of aromatic boric acid with 4-halogenated pyrazolyl ring, usually by iodination or bromation, under Suzuki−Miyaura reaction conditions. Since the cross-coupling method would be unsuitable for the synthesis of 3- and 5- position resultants. The pyrazolyl ring can be constructed by simply reacting hydrazine or substituted phenylhydrazine with 2-substituted malonaldehyde or the corresponding enaminone to yield the 4-position and the 3- or 5-position resultants, respectively[15].

Thus, we disclose our studies on the synthesis and antibacterial activity of a novel class of oxazolidinone compounds that feature a biaryl moiety in C ring position of linezolid. In the primary screening, **10f** was demonstrated as the most potent compound, and we have reported excellent *in vitro* and *in vivo* antibacterial activity of its hydrochloric acid salt form[16–17]. With a ten-fold increase, its

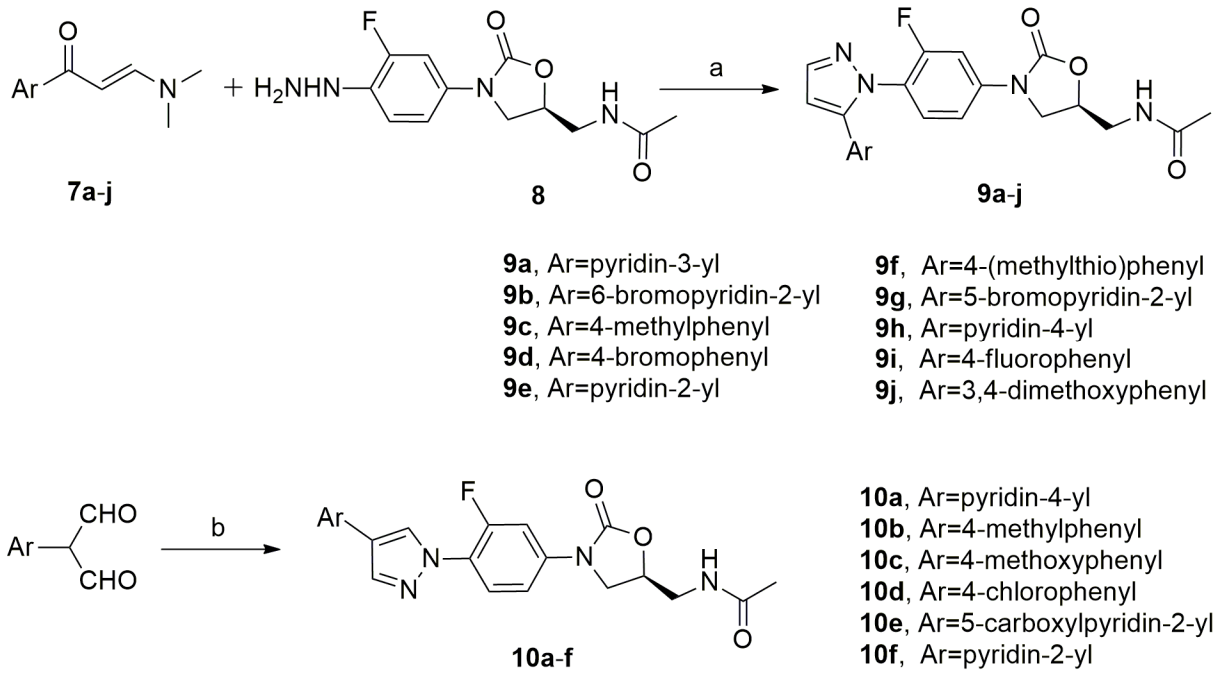
solubility still caused certain limitations in proceeding with *in vivo* experiments, although considerable pharmaceutical efforts have been made to address this issue by the addition of various adjuvants such as (2-hydroxypropyl)-β-cyclodextrin, sulfobutyl ether-β-cyclodextrin, olive oil and tween 80. In general, two more strategies could be adopted from this point to address the solubility issue: (1) structural modification by addition of soluble groups to the parent compound, which may cause activity loss and toxicity increase; (2) preparation of corresponding salts by taking advantage of the compound's basic properties. In this paper, we describe our endeavors in medicinal chemistry, acid salt screening and other relevant translational research of compound **10f**.

CHEMISTRY:

The general synthesis of compounds **9** and **10** are depicted in Scheme 1. A pyrazolyl ring formation was used to construct an aromatic–pyrazole linked system by condensing intermediate **8** with 3-(dimethylamino)-1- (aromatic-3-yl)prop-2-en-1-one (**7**) or 2-aromatic malonaldehyde to yield the final compounds **9a** to **9j** and **10a** to **10f**, respectively. Corresponding acetyl aromatic compounds were condensed with *N*, *N*-Dimethylformamide dimethyl acetal (DMF-DMA) to afford the precursor **7** under microwave conditions. The key intermediate **8** was obtained according to the reported literature with minor modifications[18]. Compound **10g** was synthesized through a long linear route depicted in Scheme 1. Intermediate 3-(dimethylamino)-2-(pyridin-3-yl)acrylonitrile **12** was prepared by condensation of 2-(pyridin-3-yl)acetonitrile and DMF−DMA without solvent under microwave irradiation as described in published procedures[19]. Compound **12** was reacted with hydrazine in the presence of acetic acid to give **13** with a high yield. The amine pyrazole compound **13** was subjected to 3, 4-difluoronitrobenzene in the presence of K_2CO_3, which yielded corresponding regio-isomeric mixture **14**. The product was notably a mixture of 3-amino and 5-amino regioisomers in a ratio of about 3 : 1, which was shown in

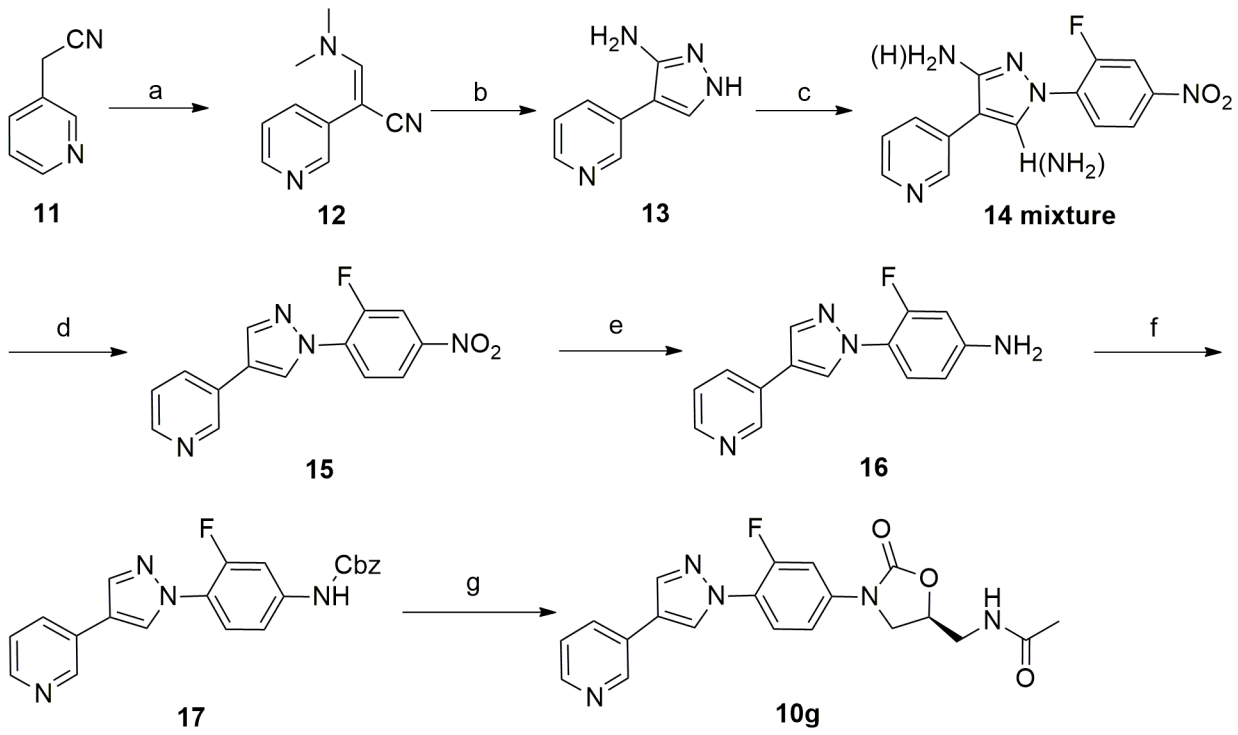
the ^{1}H NMR spectrum(Figure 2). Separation of the isomeric mixture was not required because the amino groups would be totally removed in the following reaction step. Deamination of compound **14** was carried out to yield nitro compound **15**, in which isoamyl nitrite was used as deamination agent. Then, the nitro derivatives **15** were reduced with ion powder and NH_4Cl complex in ethanol and then reacted with benzyl chloroformate to yield compound **16**. According to a general in-house method[20], the oxazolidinone ring was constructed in the final step to the target compounds **10g** by reaction of **16** with *(S)*-1-acetamido-3-chloropropan-2-yl acetate.

Scheme 1. The synthetic route of 5-substituted pyrazolylphenyl oxazolidinones 9a to 9j and 4-substituted-pyrazolylphenyloxazolidinones 10a to 10f



Reagent and conditions：(a) conc.HCl, EtOH, reflux, 31% to 50%; (b) **8**, conc. HCl, EtOH, reflux, 66% to 81%.

Scheme 2. The synthetic route of 4-substituted pyrazolylphenyloxazolidinones 10g



Reagent and conditions：(a) DMF-DMA, 100 °C, 30 min, 72%; (b) Hydrazine hydrate, acetic acid, EtOH, reflux, 24 h, 46%; (c) 3,4-difluoronitrobenzene, K_2CO_3, DMSO, 4 h, 69%; (d) Isopropyl nitrite, DMF, 75 °C, 82%; (e) Fe, NH_4Cl, EtOH, H_2O, 60 °C; (f) Cbz-Cl, K_2CO_3, DCM, overnight, 74%; (g) *t*-BuOLi, MeOH, *(S)*-1-acetamido-3-chloropropan-2-yl acetate, THF, 0 °C - r.t., 17 h, 56%.

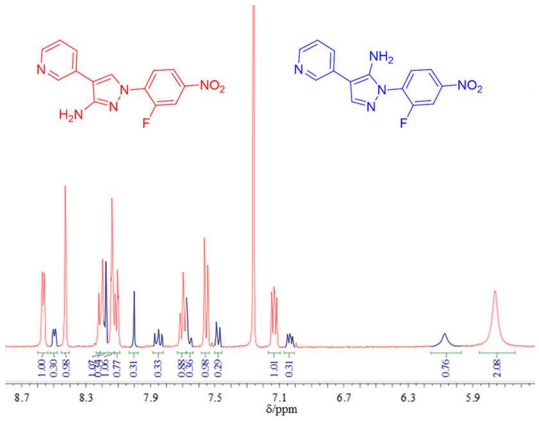
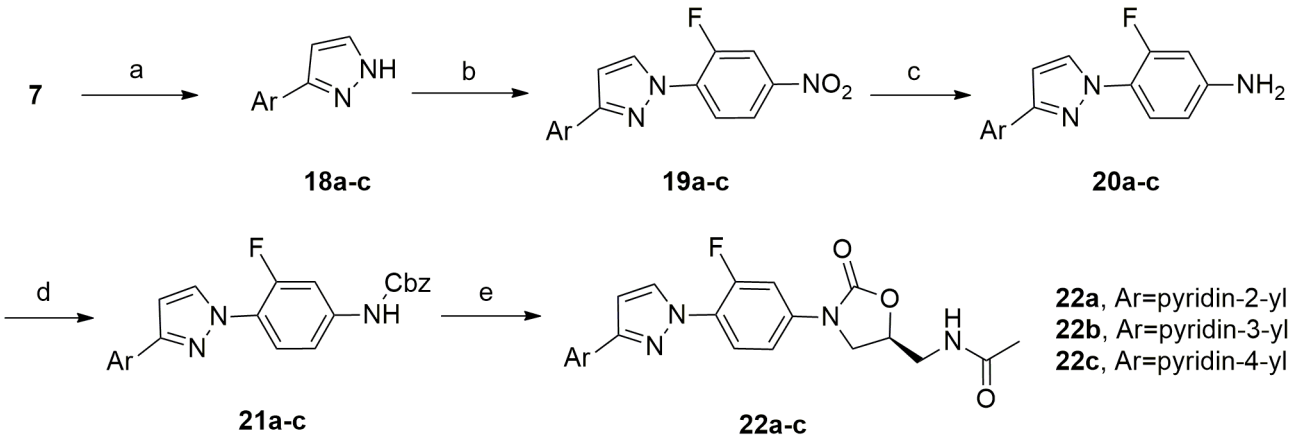


Figure 2. Component ratio determination of regioisomers of **14** by ^{1}H NMR in $CDCl_3$

Compounds **22** were obtained through a linear synthetic route starting from **7**, as depicted in Scheme 3. Starting material **7** was condensed with hydrazine hydrate in refluxed ethanol to yield intermediate **18** which was the precursor of the next nucleophilic S_NAr reaction. Referring to the

synthetic route depicted in Scheme 2, compound **18** and 3, 4-difluoronitrobenzene were reacted in the presence of K_2CO_3 to afford the nitro derivatives **19**, which were reduced with ion powder and NH_4Cl system in ethanol and successively mixed with benzyl chloroformate to yield compound **21**. In addition, target compound **22** was obtained through reaction of *(S)*-1-acetamido-3-chloropropan-2-yl acetate with compound **21** in moderate to good yields.

Scheme 3. The synthetic route of 4-substituted-pyrazolylphenyloxazolidinones 22a to 22c

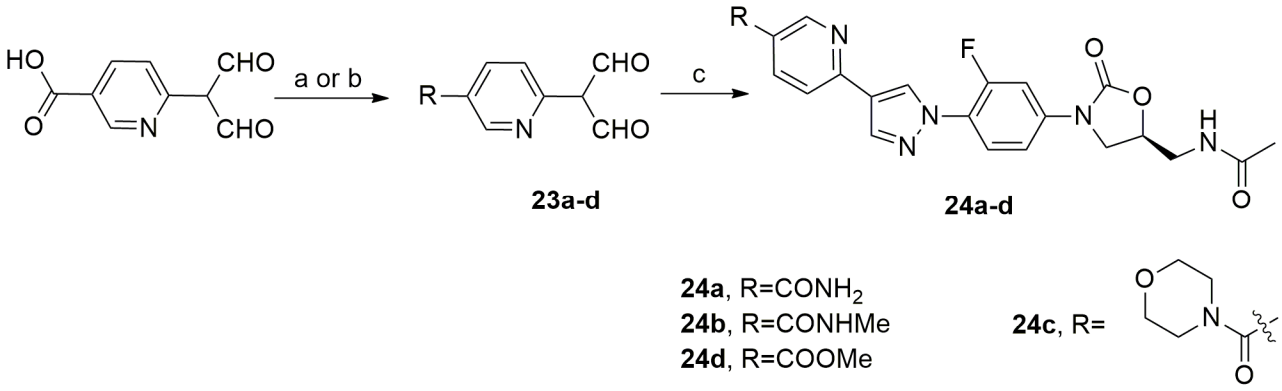


Reagent and conditions: (a) Hydrazine hydrate, EtOH, reflux, 83% to 88%; (b) 3,4-difluoronitrobenzene, DMSO, K_2CO_3, r.t., overnight, 64% to 83%; (c) Fe, NH_4Cl, EtOH, H_2O, 60 °C, 4 h; (d) Cbz-Cl, K_2CO_3, DCM, overnight, 66% to 78% (steps of c and d); (e) *t*-BuOLi, *(S)*-1-acetamido-3-chloropropan-2-yl acetate, MeOH, THF, 0 °C to r.t., 17 h, 34% to 42%.

After obtaining the most potent compound **10f**, further attempts of structural refinement were carried out to seek more potent compounds. First, several malonaldehyde compounds were obtained using amide formation reaction[21] or esterification[22], in which 5-carboxylpyridin-2-yl malonaldehyde was used as starting material (Scheme 4). Then, various substituted malonaldehyde intermediates were reacted with key intermediates **8** for the preparation of final product **24** using the same procedures for compounds **9** and **10**. Second, a series of N-containing aromatic rings was introduced to replace the pyridyl ring, which afforded the corresponding final product **28**. As illustrated in Scheme 5, the building

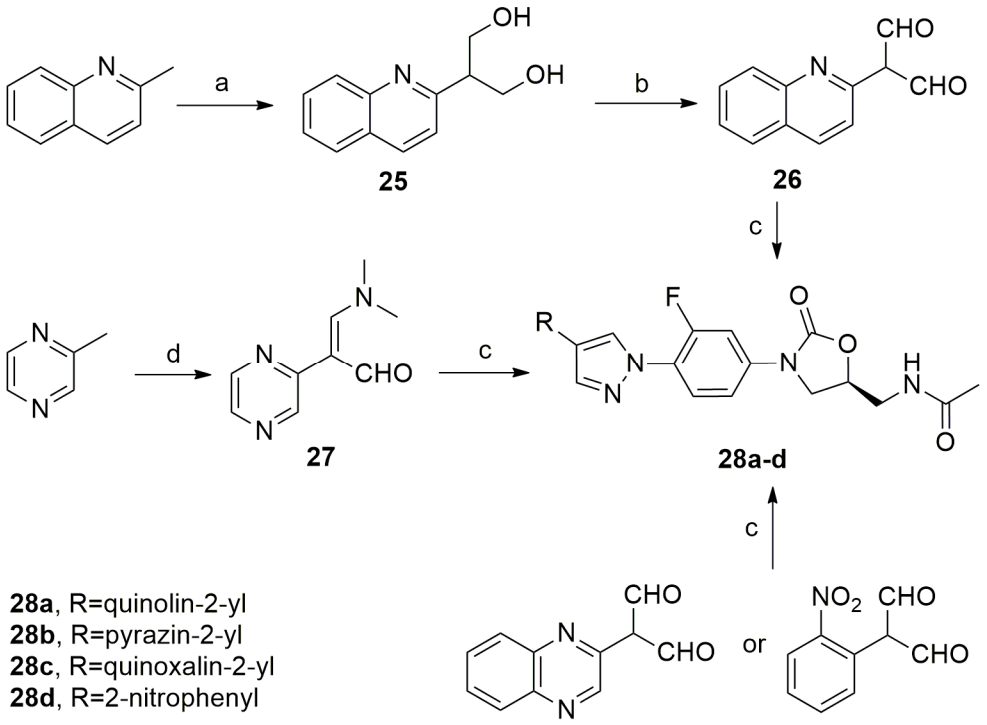
blocks for the substituted pyrazole were purchased or prepared. For the synthesis of compound **26**, oxidation of primary alcohol 2-(quinolin-2-yl)propane-1, 3-diol (**25**), synthesized through reported procedures[23], was accomplished using Parikh–Doering condition[24]. Intermediate **27**, the chemical equivalent of its malonaldehyde form, was obtained in a single step by Vilsmeier reaction with good yield[25]. Four malonaldehydes including two purchased materials or their equivalent sources were reacted with key intermediate **8** to yield the final products **28a-28d**.

Scheme 4. The synthetic route of 4-substituted pyrazolylphenyl oxazolidinones 24a to 24d



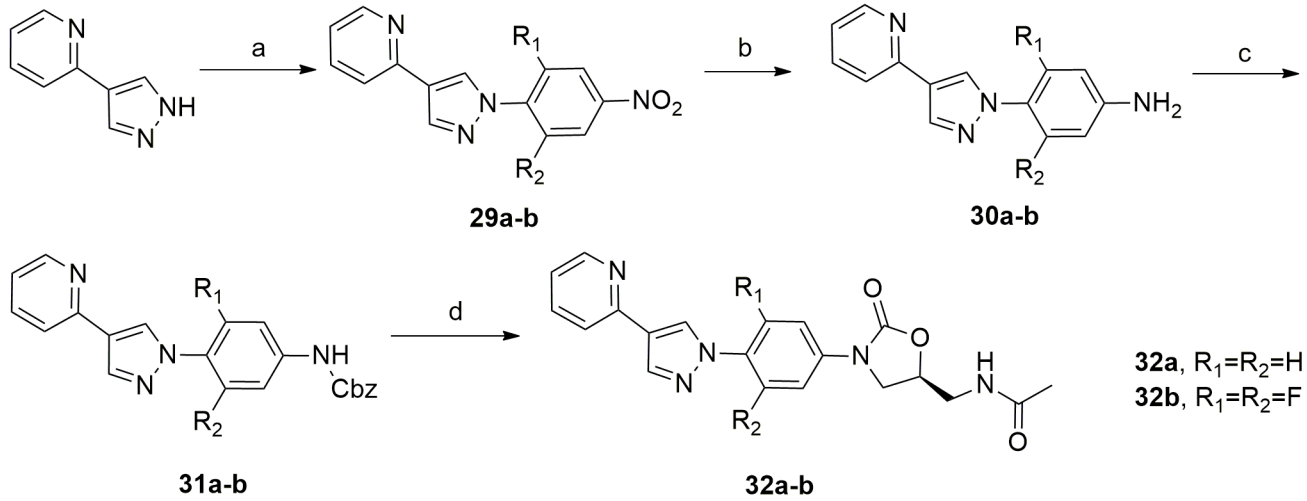
Reagent and conditions: (a) HOBt, EDCI, NH_4Cl (for synthesis of **24a**) or methylamine hydrochloride (for synthesis of **24b**) or morpholine (for synthesis of **24c**), 4-methylmorpholine, DMF, 60 °C, overnight, 47% to 65%; (b) MeI, K_2CO_3, DMF, 60 °C, 52%; (c) conc. HCl, **8**, EtOH, reflux, 4 h, 68% to 75%.

Scheme 5. The synthetic route of 4-substituted pyrazolylphenyl oxazolidinones 28a to 28d



Reagent and conditions: (a) 2-methylquinoline, formalin, 130 °C, 7 h, 45%; (b) $Py{\cdot}SO_3$, DMSO, Et_3N, methylene chloride, 0 °C to r.t., 2 h, 40%; (c) **8**, conc.HCl, EtOH, reflux 4h, 57% to 64%; (d) DMF, $POCl_3$, 2-methylpyrazine, 0°C, 1 h, 60°C, overnight, 49%.

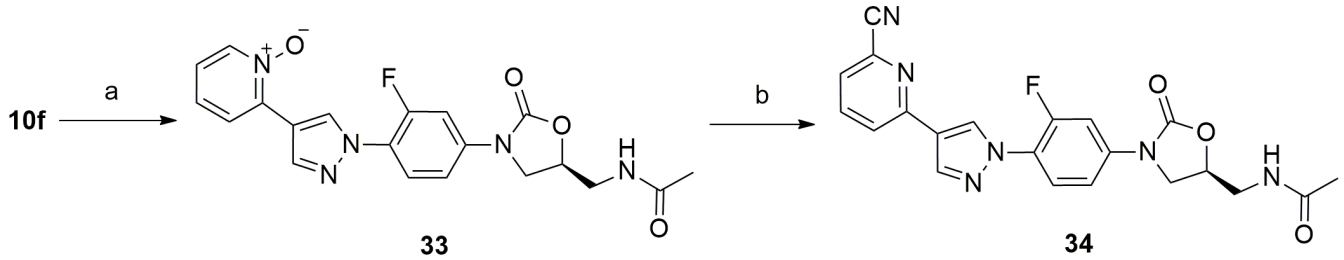
Scheme 6. The synthetic route of 4-substituted pyrazolylphenyl oxazolidinones 32a-b



Reagent and conditions: (a) 4-fluoronitrobenzene (for synthesis of **29a**) or 3,4,5-trifluoronitrobenzene (for synthesis of **29b**), K_2CO_3, DMSO, 72% to 87%; (b) Fe, NH_4Cl, EtOH, H_2O, 60 °C, 4h; (c) Cbz-Cl, K_2CO_3, DCM, overnight, 71% to 78%; (d) *t*-BuOLi, *(S)*-1-acetamido-3-chloropropan-2-yl acetate, MeOH, THF, 0 °C to r.t., 17 h, 39% to 47%.

Compounds **32a** and **32b** were synthesized to determine whether or not the fluorine atom affects antibacterial activity. As depicted in Scheme 6, a linear synthetic route was adopted to yield the final compound **32**. Nitro derivatives **29** were obtained through a nucleophilic S_NAr reaction, which used 2-(*1H*-pyrazol-4-yl)pyridine and corresponding fluoronitrobenzenes as starting materials. Target compounds **32** were synthesized in moderate yields through the route described in Scheme 2.

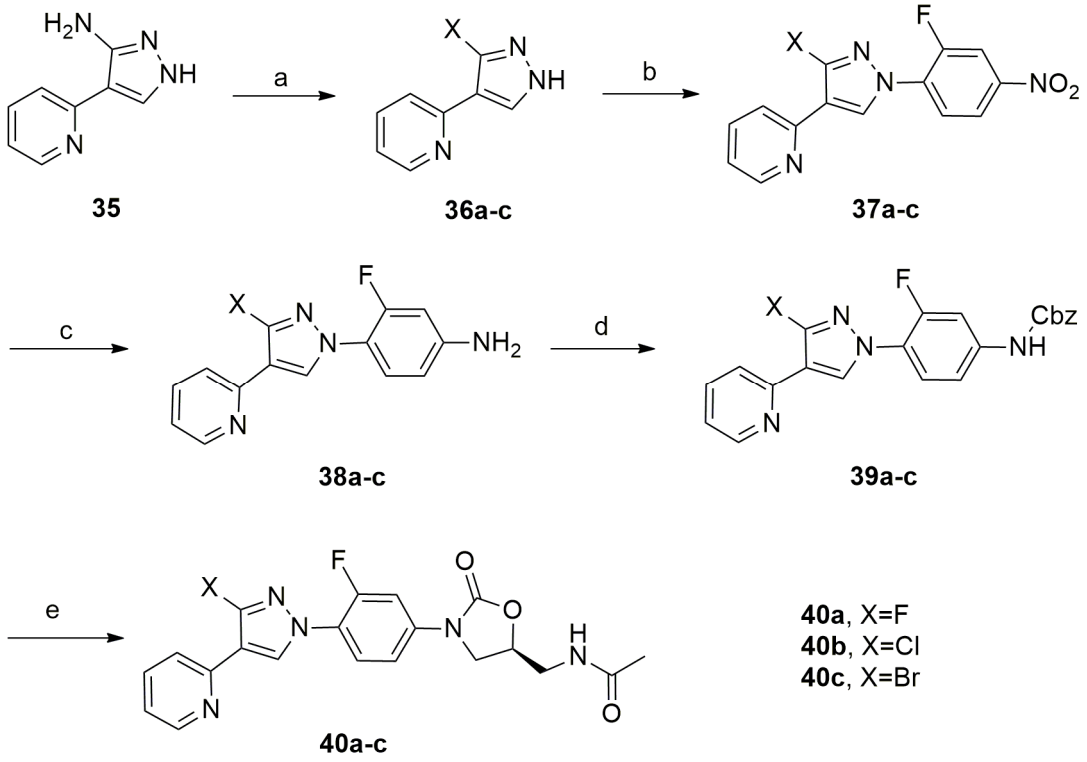
Scheme 7. The synthetic route of 4-substituted pyrazolylphenyl oxazolidinones 33 and 34



Reagent and conditions: (a) mCPBA, DCM, K_2CO_3, r.t., 18 h, 71%; (b) TMSCN, dimethylcarbamyl chloride, DCM, r.t., 48 h, 46%.

Meanwhile, to improve the aqueous solubility, the cyano group was intended to be introduced into pyridyl ring (Scheme 7). The N atom of pyridine ring of **10f** was oxidized by mCPBA to give compound **33**[26]. Then, compound **33** was subjected to TMSCN condition to afford compound **34**[27].

Scheme 8. The synthetic route of 4-substituted-pyrazolylphenyl oxazolidinones 40a to 40c

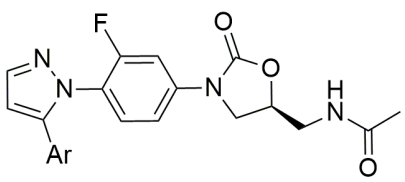


Reagent and conditions: (a) $NaNO_2$, HCl, CuCl, or HBr, CuBr or HBF_4, H_2O, 5 h, 25% to 31%; (b) 3,4-difluoronitrobenzene, K_2CO_3, DMSO, 4 h, 82% to 89%; (c) Fe, NH_4Cl, EtOH, H_2O, 60 °C; (d) Cbz-Cl, K_2CO_3, DCM, overnight, 62% to 71%; (e) *t*-BuOLi, *(S)*-1-acetamido-3-chloropropan-2-yl acetate, MeOH, THF, 0 °C to r.t., 17 h, 22% to 36%.

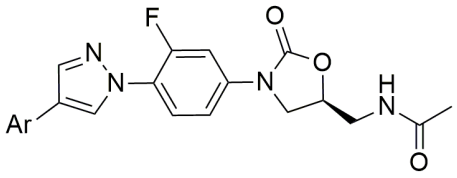
Based on **10f**, a series of compounds was synthesized to determine whether one halogen atom substituted at the pyrazolyl ring could affect antibacterial activity. As depicted in Scheme 8, target compounds **40** were also synthesized through a relatively long linear synthetic route. Intermediate **35** were prepared in high yield as previously described[20]. Sandmeyer condition was used to introduce halogen atom to the pyrazoyl ring and produce chlorinated **36b**[28] or brominated **36c**[29], whereas fluoroaromatic **36a** was obtained through Balz–Schiemann reaction[30]. Halogenated compounds **36** were then reacted with 3,4-difluoronitrobenzene in the presence of K_2CO_3 to yield the corresponding nitro derivatives **37**. As shown in Scheme 2, three sequential transformations were performed using the nitro derivatives as starting materials to yield the final compounds **40**.

RESULTS AND DISCUSSION

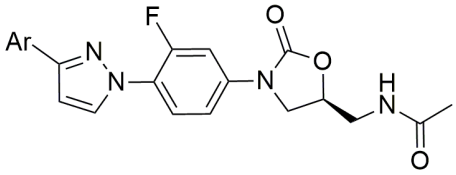
Table 1. *In Vitro* Antibacterial Activity of Substituted Pyrazolylphenyl Oxazolidinones



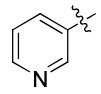
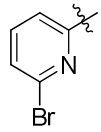
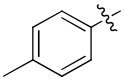
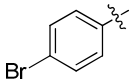
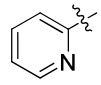
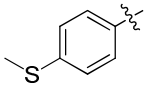
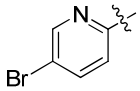
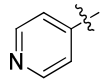
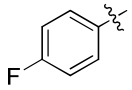
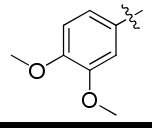
9a-j

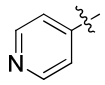
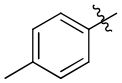
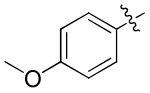
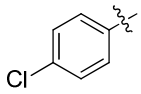
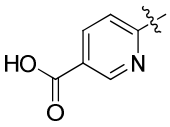
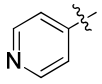


10a-g



22a-c

Compd	Ar	MIC [a] (μg/mL)							
		S.a [b]	*S.a* [c]	*S.a* [d]	*S.a* [e]	*S.a* [f]	*S.a* [g]	*E.c* [h]	*P.a* [i]
9a		8	16	8	8	16	16	>32	>32
9b		16	16	32	32	32	16	>32	>32
9c		16	16	32	32	32	32	>32	>32
9d		16	8	16	16	8	16	>32	>32
9e		8	16	16	16	16	32	>32	>32
9f		16	32	32	16	32	32	>32	>32
9g		8	4	8	8	8	8	>32	>32
9h		4	4	4	4	4	4	>32	>32
9i		32	16	32	32	32	32	>32	>32
9j		16	8	16	8	16	16	>32	>32

10a		4	4	4	4	4	8	>32	>32
10b		4	8	8	8	8	8	>32	>32
10c		4	8	4	8	8	8	>32	>32
10d		8	4	8	4	8	8	>32	>32
10e		8	8	4	8	4	8	>32	>32
10f		1	1	1	1	1	2	>32	>32
10g		4	4	4	4	4	4	>32	>32
22a		4	4	8	4	8	8	>32	>32
22b		2	2	2	2	2	2	>32	>32
22c		4	4	4	4	8	8	>32	>32
LZN	-	2	1	2	2	2	2	>32	>32

Abbreviations: [a] MIC is defined as the minimum concentration of a compound that inhibits growth by 99%. Identical values were obtained for each compound in three replicates by visual investigation as stated in the experimental section; [b] MSSA ATCC25923; [c] MSSA ATCC29213; [d] MSSA ATCC6538; [e] MSSA CMCC26003; [f] MRSA ATCC33591; [g] MRSA ATCC43300; [h] *Escherichia coli* ATCC25922; [i] *Pseudomonas aeruginosa* ATCC27853.

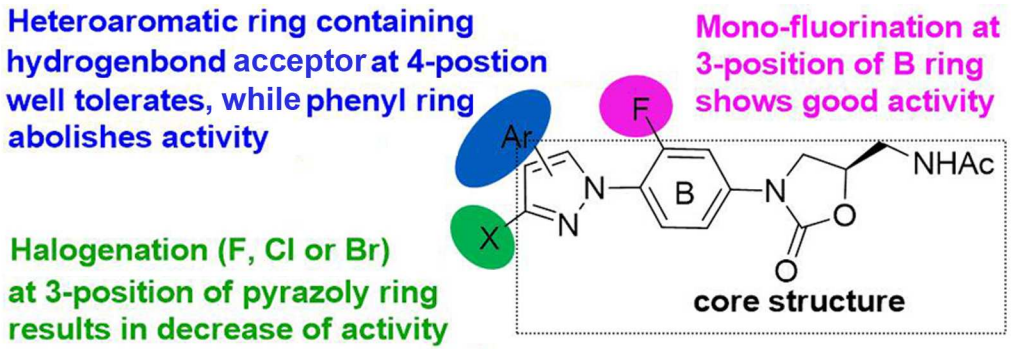


Figure 3. Regions subjected to separate SAR investigation

As designed, aromatic rings were introduced to the 3-, 4- and 5-position of the pyrazolyl ring to generate compounds **22a** to **22c, 10a** to **10g** and **9a** to **9j**, respectively, which are responsible for the generation of three types of C ring moieties (with different space projections). All oxazolidinone analogues prepared were tested for their *in vitro* activity against various Gram-positive and Gram-negative bacteria. MIC values were determined using the standard agar dilution method. The results of these studies are summarized in Table 1 and Table 2.

Compounds with aromatic ring substituents at the 5-position of the pyrazolyl ring generally showed weak activity against all test bacteria, whereas strong activity was observed when aromatic rings were connected to the 3- or 4-position of the ring. Thus, we focused on **10** and **22**, where the aromatic substituents of C ring moieties were similar to those in compounds **9**. Compounds bearing pyridyl groups (**10a**, **10f**, and **10g**) displayed stronger activity than those bearing phenyl or substituted phenyl groups (**10b**, **10c** and **10d**). These results implied that the N atom attached at different positions of the pyridyl ring may be the hydrogen bond acceptor responsible for the activity. Then, the 3- position of the pyrazolyl ring was introduced with heterocyclic substituents such as 2-pyridyl, 3-pyridyl and 4-pyridyl to yield compounds **22**. This set of oxazolidinones showed moderate to good antibacterial activity. Therefore, we speculated that the N atom of the pyridyl group may contribute to the biological activity possibly because of its participation in hydrogen bonding.

After achieving the most potent compound **10f**, we conducted extensive structure–activity relationship (SAR) studies on pyrazolyl functionality and B-ring. We selected **10f** as the parent structure to remain unchanged.

Fluorination is beneficial in many cases[31, 32]. Thus, we assessed the activity of fluorinated compounds (**32a** and **32b**). As illustrated in Table 2, in B-ring subtypes, **10f** was more potent than **32a** and **32b**. Thus, the monofluorinated derivative was superior to the fluoride-free or difluorinated ones.

Halogen atoms, including fluorine, chlorine and bromine atoms, were introduced at 3- position of pyrazole ring of compound **10f** through Sandmeyer reactions. However, the halogenated analogs **40a** to **40c** had two-fold to four-fold decrease in antibacterial activity than **10f** (Table 2). We speculated that the added hydrophobicity and steric bulk of halogens on the pyrazole ring affects the antibacterial activity.

Further development and potential applications of **10f** are hindered by its low aqueous solubility. Disruption of molecular planarity or addition of water-soluble group was considered to improve the aqueous solubility of **10f**[33]. Compound **34,** in which a cyano group was attached at the *ortho*-postion of the pyridyl group, was synthesized and evaluated for its antibacterial activity. Its synthetic intermediate **33** was also evaluated for comparison (Table 2). However, compounds **33** and **34** exhibited approximately two-fold to four-fold lower antibacterial activity than **10f**.

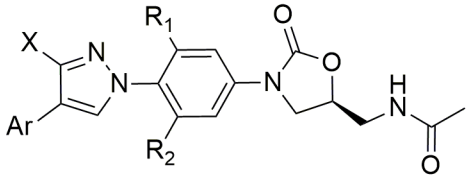
Conversely, a carboxyl group was introduced at the 5-position of the pyridyl ring for another optimization endeavor. However, the resultant compound **10e** showed lower antimicrobial activity than **10f**. The extremely poor solubility of the compound in both organic and aqueous solvents may be the key underlying reason. Thus, a series of analogues was designed and synthesized by transforming carboxyl group through amidation and esterification. Alterations to these moieties or groups can directly

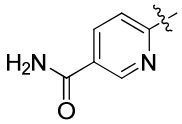
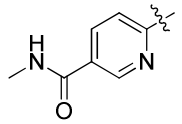
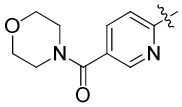
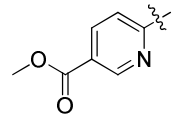
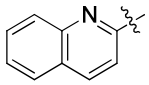
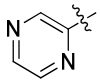
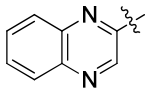
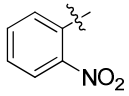
improve the solubility of the compound to some extent and they supply potential linking methods for further derivation, such as preparation of activity-based profiling probes for identification of biological targets. This particular modification was beneficial, as shown in Table 2. The esterified compound (**24d**) showed stronger activity than linezolid, with MIC values ranging from 0.5 μg/mL to 1 μg/mL. In addition, amidated compounds bearing a relatively small substituent (compounds **24a** and **24b**) at this site displayed good to moderate *in vitro* antibacterial activity compared with that of linezolid. However, morpholine ring substitution at the same site resulted in reduced activity (compound **24c**).

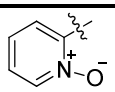
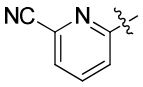
On the basis of the above-mentioned hydrogen bonding for structural optimization, several other heteroaromatic moieties were selected to mimic the interactions of pyridyl rings in accordance with bioisosterism theory. For this purpose, the core structure was remained unchanged, and the 4-position of the pyrazolyl ring was modified. In consequence, a series of Ar-substituted pyrazolyl oxazolidinone derivatives at the 4-position were synthesized and evaluated for their *in vitro* antibacterial activity, in which the Ar substituents include pyrazinyl, quinolyl and quinoxalinyl groups. Among the aforementioned analogues, as shown in Table 2, **28b** and **28c** displayed excellent antibacterial activity against all tested Gram-positive bacteria, while **28d** exhibited a reduced activity with MIC values of only 2 to 4 μg/mL. Compound **28a** with a quinolyl group to replace the pyridyl ring appeared unexpectedly ineffective, which is far beyond to our prediction and is under further investigation. By contrast, compound **28c**, which features a quinoxalinyl group that contains an extra nitrogen atom at the 4-position of the quinolyl group, exhibited excellent activity. The extra nitrogen atom, which was the unique distinction between two oxazolidinones, was considered to be important for the action. Far from being exhaustive, this initial exploration suggests that the hydrogen bonding responsible for activity is more pronounced when the six-member aromatic ring contains more hydrogen acceptor N atoms, which

allow greater degrees of freedom and probabilities to approach the site. Consequently, this region can tolerate a relatively wide range of chemical space.

Table 2. *In Vitro* Antibacterial Activity of Substituted Pyrazolylphenyl Oxazolidinones



Compd	Ar	X	R_1	R_2	MIC[a] (μg/mL)							
					S.a[b]	*S.a*[c]	*S.a*[d]	*S.a*[e]	*S.a*[f]	*S.a*[g]	*E.c*[h]	*P.a*[i]
24a		H	F	H	1	1	1	1	1	1	>32	>32
24b		H	F	H	2	2	2	2	2	2	>32	>32
24c		H	F	H	8	4	4	8	8	4	>32	>32
24d		H	F	H	0.5	1	1	1	1	1	>32	>32
28a		H	F	H	>32	>32	>32	>32	>32	>32	>32	>32
28b		H	F	H	1	1	1	1	0.5	1	>32	>32
28c		H	F	H	1	1	1	1	0.5	1	>32	>32
28d		H	F	H	4	2	2	4	4	4	>32	>32
32a		H	H	H	8	8	8	8	8	4	>32	>32
32b		H	F	F	8	4	4	8	8	4	>32	>32

33		H	F	H	4	2	2	4	2	4	>32	>32
34		H	F	H	4	4	4	8	4	8	>32	>32
40a		F	F	H	4	2	4	4	4	4	>32	>32
40b		Cl	F	H	4	2	4	4	8	8	>32	>32
40c		Br	F	H	8	4	8	8	8	8	>32	>32
10f		H	F	H	1	1	1	1	1	2	>32	>32
LZN	-	-	-		2	1	2	2	2	2	>32	>32

Abbreviations: [a] MIC is defined as the minimum concentration of a compound that inhibits growth by 99%. Identical values were obtained for each compound in three replicates by visual investigation as stated in the experimental section; [b] MSSA ATCC25923; [c] MSSA ATCC29213; [d] MSSA ATCC6538; [e] MSSA CMCC26003; [f] MRSA ATCC33591; [g] MRSA ATCC43300; [h] *Escherichia coli* ATCC25922; [i] *Pseudomonas aeruginosa* ATCC27853.

To explore the possible interaction modes of teraryl oxazolidinone with *D. radiodurans* 50S ribosomal subunit (PDB code: 3DLL), a molecular flexible docking study was conducted, and the results are shown in Figure 4. As depicted, the C-5 side chains of both compounds (**28b** and **10f**) went deep into the pocket, which was inconsistent with binding mode of linezolid (C) for the hydrogen bond formed between the hydrogen atom of the amide group and residue C2505. A π stack interaction may occur between residue U2585 and pyridyl ring or pyrazinyl ring, while a hydrogen bond, as depicted in Figure 4-C, formed between residue U2585 and oxygen atom of morpholine ring of Linezolid. The nitrogen atom of the pyridyl ring of **10f** provided hydrogen bond interaction with residue C2067 (Figure 4B), as well as the *ortho*-position nitrogen atom of the pyrazinyl ring of **28b**. However, the pyrazinyl

ring of **28b** rotated to offer extra hydrogen bond interaction between the *meta*-position nitrogen atom and residue A2439 (Figure 4A). This hydrogen bond may be the best explanation for its improved *in vitro* activities. In addition, another hydrogen bond formed between residue C2066 and N-2 atom of pyrazolyl ring of both compounds.

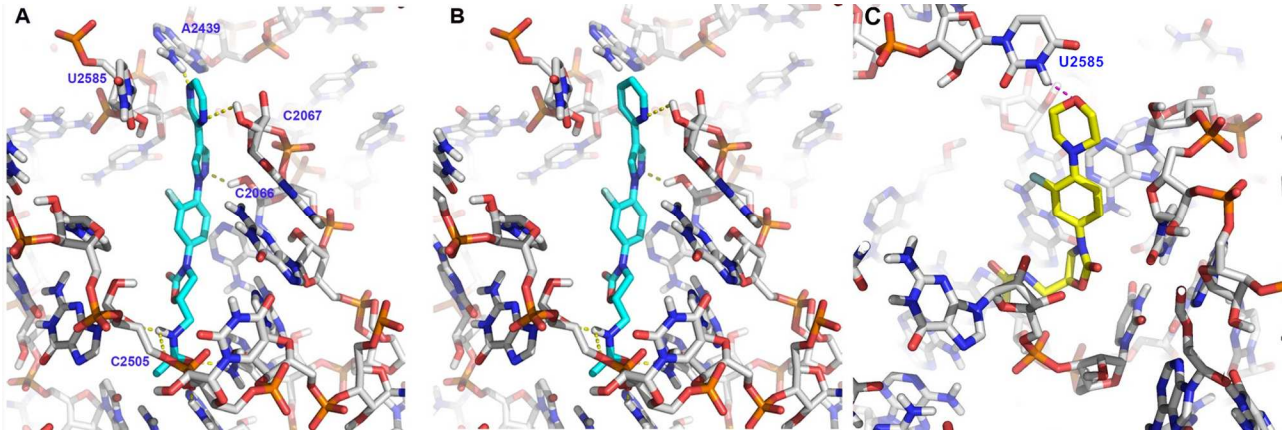


Figure 4. *In silico* docking models of the compound–target complex. Compound **28b** (A, colored by cyan), **10f** (B, colored by cyan) and Linezolid (C, colored by yellow) interacting with residues in the binding site of 50S ribosomal subunit.

Table 3. Mean CC_{50} of Selected Compounds and Linezolid in HEK 293 Cells, L02 Cells, THP-1 Cells and K562 Cells after Incubation for 48 h

Compd	CC_{50} (μM; μg/mL[a])			
	L02	HEK 293	THP-1	K562
10f	72.9 ± 3.2; 28.8 ±1.2	97.3 ± 4.5; 38.5±1.8	> 160; > 63.3	> 160; > 63.3
24a	> 160; > 70.1	24.6 ± 3.6; 10.1 ±1.6	> 160; > 70.1	> 160; > 70.1
28b	71.1 ± 4.3; 28.2 ± 1.7	69.2 ± 4.1; 27.4 ± 1.6	> 160; > 63.4	> 160; > 63.4

28c	52.4 ± 3.8;	> 160;	> 160;	> 160;
	23.4 ± 1.7	> 71.4	> 71.4	> 71.4
LZN	149.5 ± 8.6;	72.4 ± 5.5;	> 160;	> 160;
	50.4 ± 2.9	24.4 ± 1.9	> 54	> 54

[a] For better reading, μg/mL values for cytotoxicity are also concluded.

At an early stage, the safety profile identification of compounds could be conducted by cytotoxicity analysis performed with several mammalian cells, like K562 and HEK 293 cells, which could partially reflect *in vitro* myelosuppression ability[34]. Thus, with several potent compounds available, we evaluated their cytotoxicity on HEK 293 and K562 cell lines, as well as with L02 and THP-1 cell lines. All CC_{50} values were calculated after 48 h incubation. As shown in Table 4, for HEK 293 and K562 cell lines, all tested compounds displayed low cytotoxicity, except for compound **24a**, which inhibited HEK 293 cell lines at a relative high degree with a CC_{50} value of 24.6 μM. Then, THP-1 and L02 cell lines were not significantly inhibited, and all tested compounds expressed a different degree of cytotoxicity against L02 cell line. In addition, linezolid presented low cytotoxicity to the tested cell lines. Compounds **10f** and **28b** showed satisfactory cytotoxicity against all tested cell lines and were selected for further investigation.

Table 4. Characterization of Various Salts of Selected Oxazolidinone Compounds

Salts	Characterization			
	size (nm)[a]	aqueous solubility (mg/mL)[b]	appearance[c]	pH[d]
10f	-	0.3	-	-
10f·HBr	898	0.6	pale-yellow solid	2.19

10f·H_2SO_4	2248	1.8	pale-yellow solid	1.77
10f·HCl	264	4.3	pale-yellow solid	2.04
10f·TsOH	971	1.1	white solid	1.89
10f·H_3PO_4	0.7	47.1	yellow solid	2.75
10f·Oxalic acid	668	2.0	white solid	2.10
28b	-	0.05	-	-
28b·H_3PO_4	684	0.1	pale-yellow solid	2.01
28b·HCl	687	0.06	pale-yellow solid	3.1
Linezolid·HCl	-	-	viscous solid	-
Linezolid	-	2.7	-	-

[a]The particle size distribution of prepared solution was determined by a Malvern Nano-ZS 90 laser particle size analyzer after equilibration for 2 min. [b]In mg/mL, determined in water, at 30 °C. [c]Visual observation of products after filtration at room temp (within 12 h). [d] pH determination was carried out for the saturation solution of corresponding salts with pH electrode, whereas pH of **10f·H_3PO_4** was detected at concentration of 4.71 mg/mL.

Considering the basicity of the pyridyl ring in compound **10f**, it was arbitrarily prepared in our previous work as its hydrochloric acid salt with which *in vivo* experiments at low dosage, like pharmacodynamics research, could be accomplished. Its hydrochloric acid salt could form nanoassemblies in aqueous solution by self-assembly method in pure water, with an average particle size of 264.6 nm[17][17], and observed solubility was elevated from 0.3 mg/mL to 4 mg/mL. This improved solubility still did not meet the demand of drug administration in the toxicology evaluation and other *in vivo* experiments even aided by a variety of pharmaceutical adjuvant. In light of the results, to further improve the aqueous solubility, a panel of common acids were screened for the salts preparation of

compounds **10f** and **28b**, including hydrochloric acid, *p*-toluene sulfonic acid, sulfuric acid, oxalic acid, hydrobromic acid and phosphoric acid. All these salts were examined for aqueous solubility, as well as the particle size and pH value of their aqueous solution. As illustrated in Table 4, most of the salts appeared to be aggregated and featured poor solubility in water, except for **10f** phosphate, with solubility of 47.1 mg/mL. The solubility of **10f** phosphate appeared to be significantly enhanced from that of its base form and was higher than that of linezolid. The observed particle size is extremely tiny (0.7 nm) which indicates the real solution in water in form of single molecule and meets the requirement of intravenous injection development. However, efforts toward enhancing the solubility of **28b** through this method failed even though phosphate appeared as the most soluble salt, with an aqueous solubility of 0.1 mg/mL.

Table 5. hERG K^+ Channel Inhibition of Selected Compounds

Compd	hERG K^+ Channel Inhibition IC_{50} (μM)[a]
10f	> 40
28b	> 40
Linezolid	> 40
Cisapride	0.13

[a] Measured in hERG-expressing CHO cells using Qpatch 16X assay.

In most cases, noncardiovascular drugs with high liability to block the human ether-à-go-go related gene (hERG) K^+ channel and delayed cardiac repolarization can induce QT interval prolongation, which is frequently associated with potentially lethal arrhythmias[35]. In addition, marketed drugs owning to this deficiency have been called for withdrawal. Thus, QT prolongation risk was defined as one of the major

safety concerns in the course of drug development. The *in vitro* hERG channel activity in mammalian cell lines can be tested to predict QT prolongation risk[36]. Consequently, to estimate the risk, potent compounds **10f** and **28b** were selected to evaluate their affinity for the hERG channel using Qpatch system, in which linezolid was tested for comparison. As shown in Table 5, both **10f** and **28b** displayed low hERG inhibition compared with the control drug, cisapride.

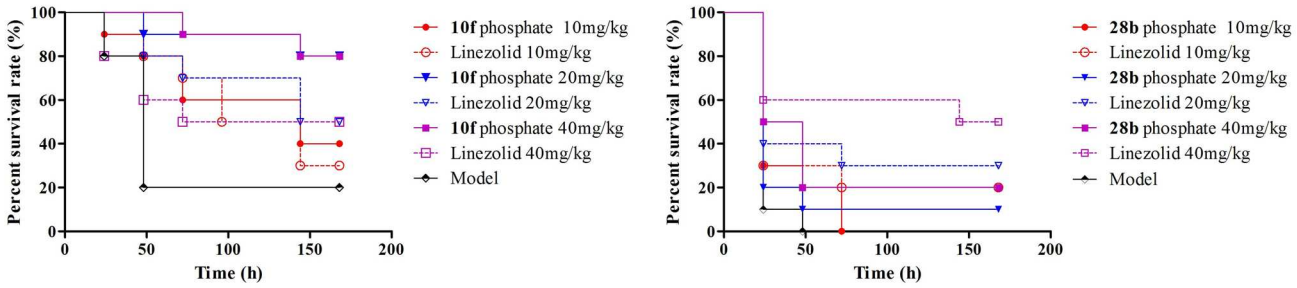


Figure 5. The survival rates of **10f** phosphate and **28b** phosphate in Mice Systemic Infection Models

Compounds **10f** phosphate and **28b** phosphate were tested in comparison with linezolid in a *staphylococcal* systemic infection model using MRSA strain ATCC33591 in mice. The dissolution of **28b** phosphate was aided by sulfobutyl ether-β-cyclodextrin. However, difficulties in administration still existed, which may result in lower bioavailability and high standard deviations in subjects' groups. As shown in Figure 5, the survival rate of compound **28b** phosphate was poorer than that of linezolid in the MRSA infection model at all dosage cohorts following intravenous administration, which may be caused by the lower exposure of **28b** resulted from the poor aqueous solubility of its phosphate. By contrast, when **10f** phosphate was intravenously administered, all dosage cohorts that treated MRSA-infected mice presented high survival rates over those treated with linezolid at the corresponding dosage. Consequently, **10f** phosphate displayed better *in vivo* activity against MRSA strain ATCC33591 than linezolid and **28b** phosphate.

Table 6. Antimicrobial Activity of 10f phosphate against 120 Clinically Isolated Strains

Strains	Compd	MIC (μg/mL)			
		MIC_{50}	MIC_{90}	MIC_{Mode}[a]	MIC_{Range}
MSSA (14)	**10f·H_3PO_4**	1	1	1	0.5-1
	Linezolid	1	1	1	1
MRSA (23)	**10f·H_3PO_4**	1	1	1	0.5-1
	Linezolid	1	1	1	0.5-1
PSSP (13)	**10f·H_3PO_4**	1	1	1	0.5-1
	Linezolid	1	1	1	0.5-1
PRSP/PISP (14)	**10f·H_3PO_4**	0.5	1	0.5	0.5-1
	Linezolid	0.5	1	0.5	0.5-1
Streptococcus	**10f·H_3PO_4**	0.25	0.5	0.25	0.25-0.5
pyogenes (15)	Linezolid	0.25	1	0.25	0.25-1
Streptococcus	**10f·H_3PO_4**	1	1	1	0.5-1
agalactiae (17)	Linezolid	1	1	1	0.5-1
VSE (12)	**10f·H_3PO_4**	1	1	1	1-2
	Linezolid	1	1	1	1-2
VRE (12)	**10f·H_3PO_4**	1	1	1	1-2
	Linezolid	1	1	1	1-2

[a]MIC_{mode} is the value that is repeated most often in the data set of MIC values against a series of bacterial isolates.

With high efficacy *in vivo*, we subjected **10f** phosphate to an antimicrobial spectrum evaluation, in which 120 Gram-positive isolate strains were used. As concluded in Table 6, **10f** phosphate displayed excellent antimicrobial activity against all test isolates, which implied that **10f** phosphate could be a

wide-spectrum anti-Gram-positive bacteria agent.

Table 7. Pharmacokinetic Parameters of Compounds 10f and 10f phosphate

Compd	route	Dose (mg/kg/day)	C_{max} (μg/ml)	t_{max} (h)	$t_{1/2}$ (h)	$AUC_{0-\infty}$ (μg.h/mL)	clearance (mL/h/kg)	F (%)
10f	*po*	10 mg	5.8	11	13	157.1	0.08	76.7
	iv	10 mg	33.1	0.12	0.9	204.8	0.05	
10f H_3PO_4	*po*	10 mg	4.2	2.4	14.7	81.7	0.15	99.1
	iv	10 mg	12.3	0.12	8.7	82.3	0.12	

The pharmacokinetic properties of **10f** phosphate and its parent compound **10f** were measured in SD rats following oral administration and intravenous injection, in which experiments (2-hydroxypropyl)-β-cyclodextrin was employed as vehicle. As illustrated in Table 7, although **10f** featured relatively poor solubility, its pharmacokinetic profiles were claimed acceptable with moderate half-life and oral bioavailability. However, its phosphate form upgraded some of PK properties, including better half-life of 14.7 h and excellent comparable oral bioavailability to linezolid of which the oral bioavailability was reported as 100%[37]. Gaining insights from these results, research works on tissue distribution and metabolism of **10f** phosphate are underway in our laboratory and will be reported in due course.

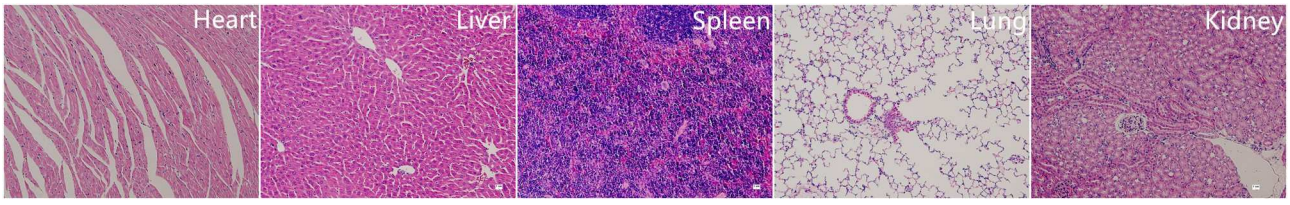


Figure 6. Histomorphological assay of main organs of BALB/c mice one week after **10f** phosphate treatment

Acute toxicity information on **10f** phosphate was determined by 7-day studies. Mice received **10f** phosphate through oral administration at doses of 448 and 896 mg/kg/day. Body weights of mice were

monitored daily. In both dosage cohorts, no evident signs of adverse results were observed in body weight, behavior or feeding. Furthermore, H&E staining to sections of the heart, liver, kidney, spleen and lung of mice in the 896 mg/kg group were observed under a microscope (Figure 6) and revealed that no histopathological changes were found in major organs after oral administration of **10f** phosphate.

Among various adverse events of oxazolidinones, hematologic toxicity, which includes anemia, thrombocytopenia, and hematocytopenia, merits particular consideration. Reversible myelosuppression has been suggested as a potential explanation for this observation[38–39].

To ascertain myelosuppressive potential of compound **10f** phosphate *in vivo*, a 4-week repeated-dose study in mice was conducted. Compound **10f** phosphate was administered orally twice daily (30 rats/group) at high dosage of 448 mg/kg/day, respectively, for 4 weeks. The linezolid comparator was dosed using a similar method at 300 mg/kg/day. As depicted in Figure 7, some mice in both **10f** phosphate and linezolid groups were deceased by day 12, but **10f** phosphate presented a relatively high survival rate of 93.3% during the test period. Notably, no apparent signs of myelosuppression including cachexia and body weight loss appeared in mice treated with both compounds. Meanwhile, blood samples were collected for assessment of hematologic toxicity and the results are concluded in Table 8.

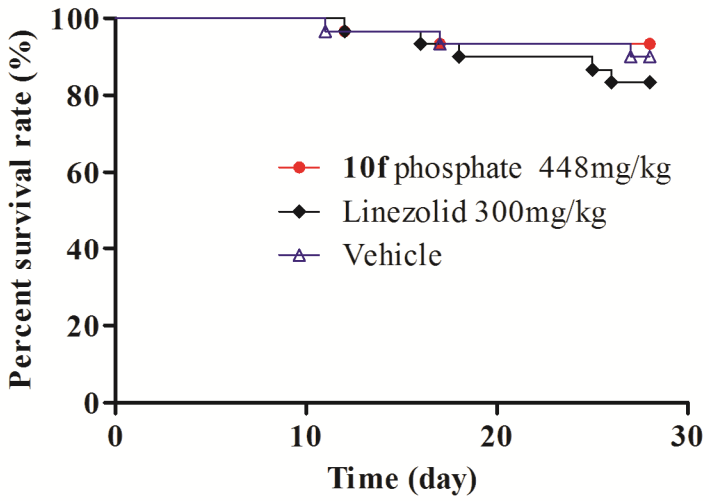


Figure 7. The survival rates of **10f** phosphate and linezolid on normal mice

Table 8. Hematology Parameters of Compound 10f phosphate in Mice[a]

Groups	WBC (10^9/L)		RBC (10^{12}/L)		PLT (10^9/L)	
	Day 14	Day 28	Day 14	Day 28	Day 14	Day 28
Normal	4.85	4.06	9.78	9.46	1728	1844
10f phosphate (448 mg/kg)	3.17	3.29	6.37	8.36	1761	1771
Linezolid (300 mg/kg)	2.16	2.88	7.99	9.03	1747	1506

[a]WBC, white blood cells; RBC, red blood cells; PLT, platelets.

As shown in Table 8, results have revealed a pronounced drop in the white blood cell (WBC) count in the 300 mg/kg/day cohort for linezolid. In contrast, **10f** phosphate induced a slight decrease in WBC count. Also, red blood cell (RBC) count was also determined. Both compounds resulted in a decrease by day 14. However, by day 28, slight rise in RBC count occurred in both cohorts, which indicates the minor myelosuppression would be reversed at the end of administration. Reductions in platelet count, which is one of the most pertinent indicators, were observed in linezolid cohorts, but not at **10f** phosphate groups, thus indicating lower thrombocytopenia potential for our compound (Table 8). The

aforementioned data indicated a minor attenuated hematological toxicity for compound **10f** phosphate in comparison with linezolid including the propensity to cause thrombocytopenia, which is denoted as a warning in the prescribing information for linezolid.

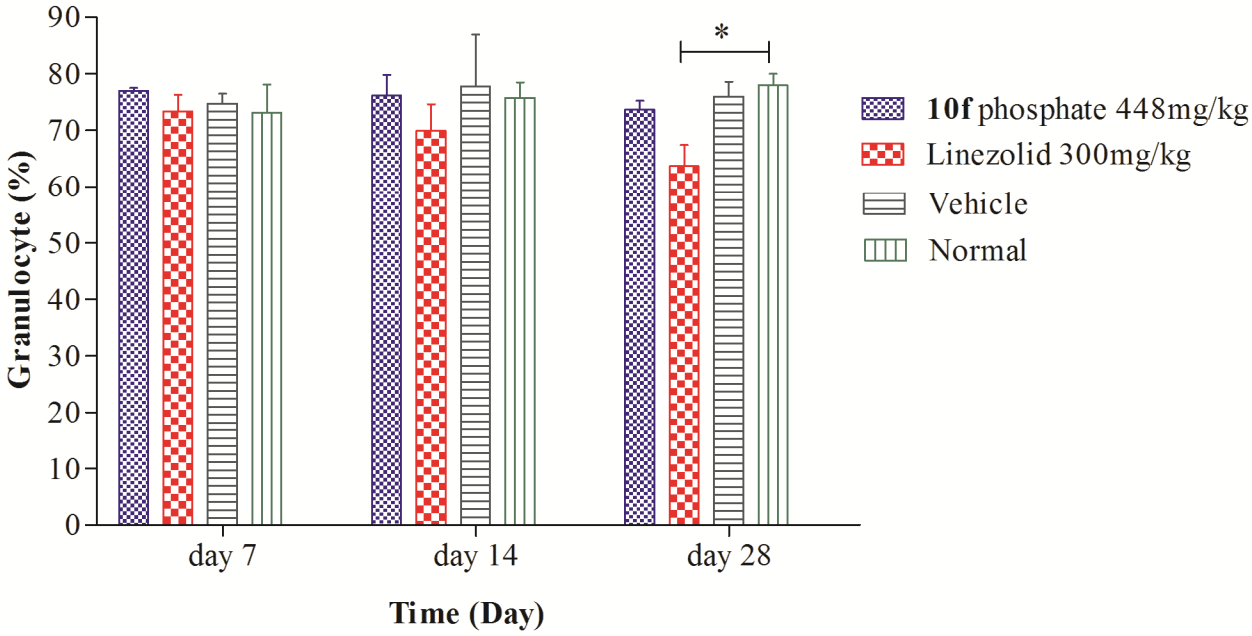


Figure 8. Granulocyte count after administration of **10f** phosphate and linezolid at days 7, 14 and 28, respectively. *, $P < 0.05$, denote significant difference compared with normal by *t*-test.

To our knowledge, myelosuppression was related to low granulocyte count. To assess bone marrow depression of **10f** phosphate, bone marrow cytological examination was accomplished and granulocytes were counted. As depicted in Figure 8, linezolid induced a downtrend of granulocytes and reached nadir at day 28, which was consistent with the result of hematology evaluation, that is, a decrease in WBC count. By contrast, no obvious decline was observed during the treatment of **10f** phosphate, which implied that a small risk factor for the leukopenia is associated with **10f** phosphate use.

CONCLUSION

An effort of critical balancing potency- and toxicology-driven structural modification was made to find a candidate that shows improved potency and reduced myelosuppression along with good PK

profile compared with linezolid. The SAR study demonstrated that compounds with heteroaromatic ring substituents at the 4-position of pyrazolyl ring presented relatively high potency. In these compounds, the hetero atoms, like nitrogen atom, were considered to meet the hydrogen bond interaction requirement of drug and U2585 residue. Analogues with no heteroaromatic ring introduced into that position were rarely potent against MSSA or MRSA. The SAR study brought about several compounds, namely, **10f**, **24a**, **24d**, **28b** and **28c**, with excellent antibacterial activity *in vitro*. Compounds **10f** and **28b** were satisfactory with low cytotoxicity on L02, HEK 293, THP-1 and K562 cells, and hERG K^+ channel. Compound **10f** phosphate with a significantly enhanced water solubility of 47.1 mg/mL was prepared, whereas the solubility of **28b** was not significantly increased. In further investigations, **10f** phosphate displayed good *in vivo* efficacy in the treatment of systematic MRSA infection mice models over linezolid, and comparable excellent PK profile to linezolid. Moreover, the compound exerted reduced myelosuppression potential compared with linezolid. Thus, the aforementioned excellence led to the promotion of **10f** phosphate as a promising antibacterial drug candidate.

EXPERIMENTAL SECTION

Preparation of salts of 10f and determination of aqueous solubility

Compound **10f** (500 mg) was suspended in acetone (100 mL) with stirring at room temperature. Then, one of the following acids (2.5 eq), which included hydrochloric acid, *p*-toluene sulfonic acid, sulfuric acid, oxalic acid, hydrobromic acid and phosphoric acid, was added into the stirring solution. The reaction mixture was agitated at room temperature overnight. The resultant solid was filtered, washed with acetone (30 mL) and dried under *vacuum* to yield the corresponding salt of **10f**.

The solubilities of the tested salts were determined by HPLC method. Stock solutions (50 μg/mL) of samples were prepared in methanol. Then 10, 20, 40, 50, 60 and 80 μL aliquots of the stock solution

were respectively injected into the HPLC system, which amounted to the calibration standards of the following concentrations, namely, 50, 100, 200, 250, 300 and 400 μg/mL, which were used to assess linearity. Calibration curves were plotted as peak area versus sample nominal concentration. A 2.5 mg aliquot of samples was added into a 1 mL centrifuge tube, and a 1 mL aliquot of pure water was pipetted into the tube. After vortexing for 30s every 5 min, which was repeated for seven times, the tube was centrifuged at 13000 r/min for 3 min. Then, these samples were kept at room temperature for 24 h. A saturated solution was obtained, and a 10 μL aliquot of the solution was injected into the HPLC system.

Molecular docking

Compounds **10f** and **28b** were docked into the *D. radiodurans* 50S ribosomal subunit crystal structure in the complex with linezolid (PDB ID: 3DLL). Preparation for the protein structure was carried out using Discovery Studio (DS) 3.0 (Accelrys, Inc., USA), which includes adding hydrogens, deleting water molecules, and assigning CHARMM like force field. Five residues including U2585, A2439, C2066, C2067 and C2055 were set as flexible, whereas other residues in the binding site were kept rigid. Compounds **10f** and **28b** were prepared and optimized using DS 3.0. A standard flexible docking protocol was carried out using GOLD program (version 5.0). The parameters were set as follows. The five residues defined above were selected as “flexible residues”. The “Number of dockings” was set to 30 without selecting an early termination option. “Detect Cavity” was turned on. The “Maximum Poses Retained” is set to 30. The GoldScore was selected as the scoring function. The other parameters were kept as default.

Minimum inhibitory concentration (MIC) testing

As described previously[17], antibacterial activity testing was performed using the broth microdilution method of the National Committee for Clinical Laboratory Standards (NCCLS M7-M4, 4th ed.). MIC

values (μg/mL) were determined on Mueller-Hinton (MH) agar medium containing dilutions of antibacterial agents ranging from 0.25 to 64 μg/mL. Tested compounds were dissolved in DMSO with a concentration of 320 μg/mL, whereas linezolid was also dissolved in DMSO as control. Serial two-fold dilutions were prepared from the stock solution with sterile water and then diluted ten-fold with MH agar medium and blood agar plate (BAP) for growth of *S. pneumoniae* to provide concentration in the range of 64 to 0.25 μg/mL. Meanwhile, growth control containing correspond concentration of DMSO (1%) for dissolving tested compound, which has been approved to be non-inhibitory on cell growth, was applied when measuring MIC. Bacterial suspensions with 0.5 McFarland standard were inoculated onto the drug-supplemented MH agar plates with a multipoint inoculation instrument and incubated at 35 °C for 18 h. MIC was defined as the lowest drug concentration that inhibits the growth of bacteria by 99%. Gram-positive organisms utilized in this study consisted of MRSA, MSSA, PSSP, PRSP/PISP, *Streptococcus pyogenes*, *Streptococcus agalactiae*, VSE and VRE. Gram-negative species included *E. coli* and *P. aeruginosa*.

Cellular Cytotoxicity

Cellular toxicity assays were carried out by MTT (3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2*H*-tetrazolium bromide) method on HEK 293 cells, L02 cells, K562 and THP-1 cells. Cells were plated into 96-well plates at a density of 1×10^4 cells per well in 100 mL of medium and grown for 48 h. The cells were then exposed to the tested compounds at different concentrations (0.25 to 160 μM) for 48 h, with linezolid as control. A 0.5% MTT solution was added to each well. After further incubation for another 4 h, formazan formed from MTT was extracted in 150 μL of DMSO for 15 min standby. Absorbance at 570 nm was then determined on a microplate reader. In brief, the mean percentage of cell survival rates relative to that of untreated cells was estimated from the data of six

individual experiments. In cytotoxicity analysis, median cytotoxic concentrations (CC_{50}) were calculated from the inhibition ratios.

Inhibition evaluation on hERG K^+ channel

Cell Culture and Transfection. A Chinese hamster ovary (CHO) cell line stably expressing hERG potassium channels was used. Cells were grown in Ham F12 (catalogue no. 11765-054, Life Technologies) supplemented with 10% (v/v) heat inactivated fetal bovine serum (FBS), 100 μg/mL hygromycin B, and 100 μg/mL geneticin (catalogue no. 10687−010, Life Technologies). Required cell density ranged between 3×10^6 and 8×10^6 cells/mL in the final suspension prior to application into the QPatch stir chamber. After extraction from the CO_2 incubator, cells are maintained in serum-free medium buffered HEPES. Cells in such condition can be used for recording only for 4 h after harvesting.

Solutions and Drugs. Cells were automatically prepared for application to chips (centrifuged and washed twice, then resuspended in extracellular solution) as described previously[40]. The intracellular solution was composed of the following (in mM): 145 NaCl, 4 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 glucose, 10 HEPES. The solution was adjusted to pH 7.4 using NaOH and possessed osmolarity of approximately 305 mOsm. The internal solution, which was adjusted to pH 7.25 using NaOH and possessed approximately 280 mOsm osmolarity, was composed of the following (in mM): 120 KCl, 5.374 $CaCl_2$, 1.75 $MgCl_2$, 10 HEPES, 5 EGTA, 4 Na-ATP. Drugs were obtained from respective manufacturers or synthesized in our laboratory.

Electrophysiology. Whole-cell recordings were performed using automated QPatch (Sophion). Cells were voltage-clamped at a holding potential of −80 mV. The hERG current was activated by depolarizing at +20 mV for 5 s, after which the current was returned to −50 mV for 5 s to remove

inactivation and observe the deactivating tail current. The maximum amount of tail current was used to determine hERG current amplitude. Compound powder will be prepared into 10 or 30 mM DMSO stock immediately before the experiments. All compounds will be freshly diluted in the external solution to the desired concentrations from compound stock in DMSO. After achieving break-in (whole-cell) configuration, the cells were recorded for 120 s to assess current stability. The voltage protocol described above was then applied to the cells every 20 s throughout the whole procedure. Only stable cells with recording parameters above threshold were allowed to enter the drug-addition procedure. External solution containing 0.1% DMSO (vehicle) was applied to cells to establish the baseline. After allowing the current to stabilize for 3 min, the compound was applied. Compound solution was added, and the cells were maintained in the test solution until the compound's effect reached a steady state or for a maximum of 3 min. Washout with external solution may be performed until current recovery reached steady state. Positive control cisapride is used in the experiments to ensure the normal response and quality of the cells. Data were analyzed using Assay Software provided by Sophion, XLFit or Graphpad Prism 6.0.

S. aureus Systemic Infection Model

Compounds **10f** phosphate and **28b** phosphate were studied in a mouse systemic infection model. BALB/c mice (SPF) weighing 20 g to 25 g were used in the study, with 10 mice in each group. Lethal systemic MRSA ATCC33591 infection was given to the mice by the injection of 0.5 mL o *S. aureus* 10^7 to 10^8 CFU/mL inoculum by intraperitoneal injection. Compounds were administered intravenously after infection. Survival rate was recorded every 12 h, and data was analyzed using the GraphPad Prism 5.0 (GraphPad Software, Inc., San Diego, CA, USA).

Pharmacokinetic Studies

Compounds **10f** and **10f** phosphate were tested in the pharmacokinetic study on Sprague–Dawley rats ((200 ± 20) g, male) that were obtained from Shanghai SLAC Laboratory Animal Co. Ltd. The animals were housed in a room with controlled temperature and humidity, and allowed free access to food and water. For studying both compounds, rats were divided into 2 groups (5 rats/group), and each group received the compounds intravenously and orally at a dose of 10 mg/kg, respectively. Blood samples of orally administrated rats were collected at 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12, 24 and 48 h post-dose (five rats/time point). The blood samples of intravenously injected rats were collected at 0.083, 0.167, 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12, 24 and 48 h post-dose and centrifuged at 3000 rpm and 4 °C for 10 min. The obtained plasma was collected and stored at –20 °C. All plasma samples were analyzed within one week after collection. Plasma concentration–time data were analyzed, and pharmacokinetic parameters were calculated.

In vivo toxicity studies

*Acute toxicity evaluation of **10f** phosphate*

To evaluate the possible side effects in **10f** phosphate-treated mice, all animals were carefully observed after administration of **10f** phosphate at doses of 448 and 896 mg/kg, including the general conditions (activity, energy, hair, feces, behavior pattern, and other clinical signs), body weight, and mortality. After sacrifice, various organs (heart, liver, spleen, lung and kidney) were harvested and fixed in 4% paraformaldehyde in PBS. These tissues were sectioned, stained with hematoxylin–eosin (H&E), and observed by two pathologists in a blinded manner.

*Repeat dose toxicity evaluation of **10f** phosphate*

10f phosphate was administered orally to mice, 15/sex/group, at 0 (vehicle control) and 448 mg/kg twice daily for 28 d. Mice were purchased from DaShuo Laboratory Animal co., Ltd. Every five

mice/sex/group were sacrificed and examined 1 d after the last dose. Blood samples were collected prior to dose initiation and on study days 14 and 28. Complete blood cell counts were performed on a Technicon H-1 instrument. Necropsy was performed on all mice.

Bone marrow smears were prepared from bone marrow expressed from a sternum. The slides were stained with Giemsa stain. These bone marrow smears were examined by light microscopy, and granulocytes were counted.

Ethics statement: All animal experiments were carried out according to the Guidelines for the Care and Use of Laboratory Animals that was approved by the Committee of Ethics of Animal Experimentation of Sichuan University.

Chemistry

All solvents and reagents were purchased from the suppliers and used without further purification. ^{1}H NMR spectra were recorded on a Bruker Avance (Varian Unity Inova) 400 MHz spectrometer in $CDCl_3$ or DMSO-d_6 with TMS as internal standard. Data for ^{1}H NMR are reported as follows: chemical shift (δ ppm), multiplicity (*s* = singlet, *d* = doublet, *t* = triplet, *q* = quartet, *m* = multiplet), integration, and coupling constant (Hz), whereas ^{13}C NMR analyses were reported in terms of chemical shift. NMR data were analyzed by using MestReNova Software. Mass spectra were recorded on a Waters Q-TOF Premier mass spectrometer. The purity of the final compounds was determined to be ≥97% by high pressure liquid chromatography (HPLC) using a Waters Alliance system (Waters Corp., Milford, MA, USA), which was equipped with performance PLUS inline degasser along with an auto-sampler and a 2998 photodiode array detector. High resolution mass spectrometry (HRMS) was performed on an Agilent LC/MSD TOF system G3250AA. Analytical thin layer chromatography (TLC) was performed on silica gel 60 F254 precoated plates (0.25 mm) from Qingdao Haiyang Inc., and components were

visualized by ultraviolet light (254 nm). Silicycle silica gel 300−400 (particle size 40−63 μm) mesh was used for all flash column chromatography experiments.

General Procedure A for the Synthesis of 5-substituted pyrazolylphenyl oxazolidinones 9a-j

To a stirred solution of compound **8** (1.0 eq) in ethanol (3 mL) was added compound **7** (1.0 eq) and 37% HCl (0.1 eq). The reaction mixture was then heated to reflux until completion determined by TLC, cooled and partitioned between DCM/H_2O. The organic layer was washed with brine and dried over Na_2SO_4. Removal of solvent gave residue which was purified by column chromatography on silica gel (mixture of methanol − ethyl acetate as eluent) to afford the desired compound **9a-j**.

(S)-N-((3-(3-fluoro-4-(5-(pyridin-3-yl)-1H-pyrazol-1-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)acetamide (9a)

Following general procedure A, the crude residue was purified by flash chromatography, using methanol−ethyl acetate, 1:100 (v/v), as eluent, to furnish **9a** as a yellow solid (121 mg, yield of 40.7%), mp 83.7-85.9°C, HPLC: 98.2%. ^{1}H-NMR(400 MHz, $CDCl_3$): δ 8.54 (d, J=4.4 Hz, 1H), 8.49 (s, 1H), 7.80 (d, J=2 Hz, 1H), 7.52 (m, 4H), 7.26 (m, 1H), 6.61 (d, J=2 Hz, 1H), 6.08 (d, J=6 Hz, 1H), 4.81 (m, 1H), 4.06 (t, J=9.2 Hz, 1H), 3.79 (m, 1H), 3.69 (m, 2H), 2.03 (s, 3H); ^{13}C-NMR (DMSO-d_6): δ 169.96, 156.99, 154.53, 153.93, 149.32, 148.00, 141.12, 140.44, 134.89, 129.72, 125.73, 123.60, 122.20, 113.85, 107.49, 105.47, 71.80, 47.14, 41.32, 22.40; HRMS: Q-TOF: 396.1473.

(S)-N-((3-(4-(5-(6-bromopyridin-2-yl)-1H-pyrazol-1-yl)-3-fluorophenyl)-2-oxooxazolidin-5-yl)methyl) acetamide (9b)

Following general procedure A, the crude residue was purified by flash chromatography, using methanol−ethyl acetate, 1:100 (v/v), as eluent, to furnish **9b** as a light yellow solid (78 mg, yield of 47.3%), mp 90.5-92.3°C, HPLC: 98.8%. ^{1}H NMR(400 MHz, $CDCl_3$): δ 7.79 (s, 1H), 7.50 (m, 3H), 7.34

(dd, J = 8 Hz, J = 1.6 Hz, 2H), 7.28 (m, 1H), 6.84 (d, J = 2 Hz, 1H), 6.15 (s, 1H), 4.82 (t, J = 3.2 Hz, 1H), 4.10 (t, J = 8.8 Hz, 1H), 3.83 (m, 1H), 3.68 (m, 2H), 2.03(s, 3H); ^{13}C NMR (DMSO-d_6): δ 170.10, 157.25, 154.80, 153.95, 148.85, 140.92, 140.45, 140.27, 139.86, 128.89, 127.13, 123.78, 121.21, 113.40, 108.16, 105.14, 71.78, 47.14, 41.32, 22.40; HRMS: Q-TOF: 496.0405, 498.0389.

(S)-N-((3-(3-fluoro-4-(5-p-tolyl-1H-pyrazol-1-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)acetamide (9c)

Following general procedure A, the crude residue was purified by flash chromatography, using methanol−ethyl acetate, 1:100 (v/v), as eluent, to furnish **9c** as a light yellow solid (133 mg, yield of 50.9%), mp 89.5-91.9°C, HPLC: 98.3%. ^{1}H NMR(400 MHz, $CDCl_3$): δ 7.74 (d, J=2 Hz, 1H), 7.52 (dd, J=12 Hz, J=2.8 Hz, 1H), 7.43 (t, J=8.8 Hz, 1H), 7.24 (m, 1H), 7.09 (m, 4H), 6.50 (d, J=2 Hz, 1H), 6.24 (s, 1H), 4.78 (t, J=2.4 Hz, 1H), 4.05 (t, J=8.8 Hz, 1H), 3.79 (m, 1H), 3.66 (m, 2H), 2.32 (s, 3H), 2.02 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 169.97, 157.21, 154.74, 153.94, 144.30, 140.72, 140.12, 137.89, 129.64, 129.19, 127.39, 126.77, 122.77, 113.70, 106.38, 105.59, 105.34, 71.79, 47.14, 41.33, 22.40, 20.68; HRMS: Q-TOF: 431.1492.

(S)-N-((3-(4-(5-(4-bromophenyl)-1H-pyrazol-1-yl)-3-fluorophenyl)-2-oxooxazolidin-5-yl)methyl)acetamide (9d)

Following general procedure A, the crude residue was purified by flash chromatography, using methanol−ethyl acetate, 1:100 (v/v), as eluent, to furnish **9d** as a light yellow solid (116 mg, yield of 44.0%), mp 96.8-98.8°C, HPLC:98.5%. ^{1}H NMR(400 MHz, $CDCl_3$): δ 7.81 (s, 1H), 7.48 (m, 3H), 7.26 (m, 2H), 7.07 (m, 2H), 6.54 (d, J= 1.6 Hz, 1H), 5.95 (d, J= 6.4 Hz, 1H), 4.80 (m, 1H), 4.07 (m, 1H), 3.70 (m, 3H), 2.04 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 169.97, 157.02, 154.56, 153.92, 143.09, 140.92, 140.28, 139.39, 131.72, 130.99, 129.60, 128.84, 121.86, 113.82, 107.04, 105.62, 105.37, 71.79, 47.14, 41.33, 22.40; HRMS: Q-TOF: 473.0625, 475.0607.

(S)-N-((3-(3-fluoro-4-(5-(pyridin-2-yl)-1H-pyrazol-1-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)acetamide (9e)

Following general procedure A, the crude residue was purified by flash chromatography, using methanol−ethyl acetate, 1:100 (v/v), as eluent, to furnish **9e** as a light yellow solid (99 mg, yield of 44.0%), mp 79.9-82.1°C, HPLC: 98.1%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.44 (d, J=4.8 Hz, 1H), 7.78 (s, 1H), 7.67 (t, J=8 Hz, 1H), 7.56 (m, 2H), 7.39 (d, J=8 Hz, 1H), 7.21 (m, 2H), 6.81 (s, 1H), 6.30 (m, 1H), 4.80 (t, J=2.4 Hz, 1H), 4.08 (t, J=9.2 Hz, 1H), 3.81 (t, J=8.4 Hz, 1H), 3.66 (m, 2H), 2.02 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 169.99, 153.97, 149.11, 148.42, 142.94, 140.65, 139.56, 137.07, 128.82, 124.14, 123.04, 122.23, 113.37, 107.69, 105.23, 104.97, 71.75, 47.18, 41.34, 22.41; HRMS: Q-TOF: 396.1477.

(S)-N-((3-(3-fluoro-4-(5-(4-(methylthio)phenyl)-1H-pyrazol-1-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)acetamide (9f)

Following general procedure A, the crude residue was purified by flash chromatography, using methanol−ethyl acetate, 1:100 (v/v), as eluent, to furnish **9f** as a yellow solid (82 mg, yield of 34.0%), mp 175.4-177.6°C, HPLC: 99.4%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.75 (s, 1H), 7.52 (dd, J=12 Hz, J=2 Hz, 1H), 7.44 (t, J=8.4 Hz, 1H), 7.26 (d, J=8.4 Hz, 1H), 7.13 (s, 4H), 6.51 (d, J=1.2 Hz, 1H), 6.22 (s, 1H), 4.79 (t, J=2.8 Hz, 1H), 4.06 (t, J=8.8 Hz, 1H), 3.79 (t, J=7.2 Hz, 1H), 3.71 (m, 1H), 3.60 (m, 1H), 2.46 (s, 3H), 2.02 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 169.97, 157.18, 154.72, 153.93, 143.83, 140.79, 140.23, 138.96, 129.64, 127.87, 125.69, 122.67, 113.75, 106.49, 105.62, 71.80, 47.15, 41.33, 22.40, 14.14; HRMS: Q-TOF: 441.1398.

(S)-N-((3-(4-(5-(5-bromopyridin-2-yl)-1H-pyrazol-1-yl)-3-fluorophenyl)-2-oxooxazolidin-5-yl)methyl)acetamide (9g)

Following general procedure A, the crude residue was purified by flash chromatography, using

methanol−ethyl acetate, 1:100 (v/v), as eluent, to furnish **9g** as a yellow solid (105 mg, yield of 47.9%), mp 92.7-95.4°C, HPLC:98.9%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.20 (d, J = 2 Hz, 1H), 7.90 (d, J = 2 Hz, 1H), 7.52 (m, 2H), 7.42 (m, 2H), 7.30 (d, J = 8 Hz, 1H), 6.61 (d, J =2 Hz, 1H), 6.17 (s, 1H), 4.82 (t, J = 2.8 Hz, 1H), 4.04 (t, J = 8.8 Hz, 1H), 3.81 (t, J = 7.6 Hz, 1H), 3.68 (m, 2H), 2.04 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 169.97, 156.87, 154.41, 153.92, 148.65, 141.12, 140.55, 139.91, 138.05, 129.66, 128.02, 125.46, 121.78, 113.92, 107.87, 105.54, 71.81, 47.14, 41.34, 22.40; HRMS: Q-TOF: 496.0401, 498.0385.

(S)-N-((3-(3-fluoro-4-(5-(pyridin-4-yl)-1H-pyrazol-1-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)acetamide (9h)

Following general procedure A, the crude residue was purified by flash chromatography, using methanol−ethyl acetate, 1:100 (v/v), as eluent, to furnish **9h** as a yellow solid (79 mg, yield of 32.1%), mp 74.5-76.3°C, HPLC: 98.7%. ^{1}H NMR(400 MHz, $CDCl_3$): δ 8.54 (d, J = 5.2 Hz, 2H), 7.80 (d, J = 2 Hz, 1H), 7.49 (m, 2H), 7.33 (dd, J = 2.4 Hz, J = 0.8 Hz, 1H), 7.12 (d, J = 6 Hz, 2H), 6.67 (d, J = 1.6 Hz, 1H), 6.22 (s, 1H), 4.81 (m, 1H), 4.08 (t, J = 9.2 Hz, 1H), 3.82 (m, 1H), 3.64 (m, 2H), 2.03 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 169.98, 156.98, 154.52, 153.94, 150.05, 141.56, 140.55, 136.73, 129.49, 122.16, 121.50, 113.92, 108.10, 105.53, 71.83, 47.16, 41.33, 22.40; HRMS: Q-TOF: 396.1476.

(S)-N-((3-(3-fluoro-4-(5-(4-fluorophenyl)-1H-pyrazol-1-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)acetamide (9i)

Following general procedure A, the crude residue was purified by flash chromatography, using methanol−ethyl acetate, 1:100 (v/v), as eluent, to furnish **9i** as a yellow solid (145 mg, yield of 42.2%), mp 111.4-112.9°C, HPLC: 98.5%. ^{1}H NMR (400 MHz $CDCl_3$): δ 7.75 (d, J = 2 Hz, 1H), 7.51 (dd, J = 12 Hz, J = 2.4 Hz, 1H), 7.45 (t, J = 8.4 Hz, 1H), 7.25 (m, 1H), 7.19 (m, 2H), 6.98 (t, J = 8.4 Hz, 2H),

6.50 (d, J = 2 Hz, 1H), 6.03 (t, J = 6 Hz, 1H), 4.80 (m, 1H), 4.06 (t, J = 8.8 Hz, 1H), 3.71 (m, 3H), 2.03 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 169.98, 163.16, 160.71, 157.10, 154.64, 153.93, 143.28, 140.78, 129.80, 126.17, 122.43, 115.66, 113.75, 106.84, 105.58, 71.80, 47.14, 41.32, 22.39; HRMS: Q-TOF: 413.1423.

(S)-N-((3-(4-(5-(3,4-dimethoxyphenyl)-1H-pyrazol-1-yl)-3-fluorophenyl)-2-oxooxazolidin-5-yl)methyl)acetamide (9j)

Following general procedure A, the crude residue was purified by flash chromatography, using methanol−ethyl acetate, 1:100 (v/v), as eluent, to furnish **9j** as a yellow solid (139 mg, yield of 42.6%), mp 153.7-156.1°C, HPLC:98.3%. ^{1}H NMR(400 MHz $CDCl_3$): δ 7.74 (d, J = 1.6 Hz, 1H), 7.52 (dd, J = 12 Hz, J = 2.4 Hz, 1H), 7.43 (t, J = 8.4 Hz, 1H), 7.25 (dd, J = 9.6 Hz, J = 2.4 Hz, 1H), 6.75 (m, 3H), 6.49 (d, J = 2 Hz, 1H), 6.19 (t, J = 8.4 Hz, 1H), 4.79 (m, 1H), 4.05 (t, J = 9.2 Hz, 1H), 3.86 (s, 3H), 3.79 (m, 1H), 3.69 (m, 4H), 3.62 (m, 1H), 2.02 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 169.98, 157.44, 154.97, 153.95, 148.84, 148.37, 144.31, 140.59, 140.16, 129.82, 122.93, 121.98, 120.05, 113.72, 111.45, 106.65, 105.65, 105.40, 71.80, 55.34, 47.18, 41.33, 22.38; HRMS: Q-TOF: 477.1557.

General Procedure B for the Synthesis of 4-substituted-pyrazolylphenyloxazolidinones

To a stirred solution of compound **8** (1.0 eq) in ethanol was added 2-aromatic malonaldehyde or its equivalent form (1.0 eq) and conc.HCl (0.1 eq). The reaction was then heated to reflux until completion of the reaction by TLC, cooled to room temperature. The solid was collected through filtration and washed well with ethanol. The resulting solid was dried under *vacuum* to afford the desired compound **10a-g, 24a-d and 28a-d**.

(S)-N-((3-(3-fluoro-4-(4-(pyridin-4-yl)-1H-pyrazol-1-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)acetami de (10a)

Following general procedure B, the product **10a** was isolated as a white solid (210 mg, yield of 66.3%), mp 203.8-205.0°C, HPLC: 99.9%. ^{1}H NMR (DMSO-d_6): δ 8.89(s, 1H), 8.56 (d, J = 5.2 Hz, 2H), 8.45 (s, 1H), 8.30 (t, J = 5.6 Hz, 1H), 7.85 (t, J = 8.8 Hz, 1H), 7.78 (d, J = 14 Hz, 1H), 7.73 (d, J = 5.2 Hz, 2H), 7.51 (d, J = 8.8 Hz, 1H), 4.78 (m, 1H), 4.19 (t, J = 8.8 Hz, 1H), 3.81 (t, J = 7.2 Hz, 1H), 3.45 (t, J = 5.2 Hz, 2H), 1.85 (s, 3H); ^{13}C-NMR (DMSO-d_6): δ 170.01, 154.61, 153.98, 152.15, 150.12, 139.05, 138.92, 129.73, 125.37, 122.70, 121.28, 119.83, 114.00, 106.16, 71.82, 47.20, 41.34, 22.42; HRMS: Q-TOF: 396.1469.

(S)-N-((3-(3-fluoro-4-(4-p-tolyl-1H-pyrazol-1-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)acetamide (10b)

Following general procedure B, the product **10b** was isolated as a white solid (240 mg, yield of 69.5%), mp 221.5-222.8°C, HPLC: 99.8%. ^{1}H NMR (DMSO-d_6): δ 8.59(s, 1H), 8.30(t, J = 5.6 Hz, 1H), 8.23(s, 1H), 7.84 (t, J = 9.2 Hz, 1H), 7.77 (d, J = 13.6 Hz, 1H), 7.61 (d, J = 8.0 Hz, 2H), 7.49 (d, J = 8.8 Hz, 1H), 7.22 (d, J = 8 Hz, 2H), 4.78 (m, 1H), 4.19 (t, J = 9.2 Hz, 1H), 3.80 (t, J = 7.2 Hz, 1H), 3.45 (t, J = 4.8 Hz, 2H), 2.32 (s, 3H), 1.85 (s, 3H). ^{13}C NMR (DMSO-d_6): δ 170.00, 154.42, 153.99, 151.96, 138.41, 135.77, 129.41, 128.70, 127.54, 125.26, 123.76, 120.56, 120.08, 114.00, 106.21 71.79, 47.19, 41.34, 22.42, 20.71. HRMS: Q-TOF: 431.1497.

(S)-N-((3-(3-fluoro-4-(4-(4-methoxyphenyl)-1H-pyrazol-1-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)acetamide (10c)

Following general procedure B, the product **10c** was isolated as a white solid (190 mg, yield of 80.7%), mp 226.1-227.8°C, HPLC: 98.7%. ^{1}H NMR (DMSO-d_6): δ 8.53 (d, J = 1.6 Hz, 1H), 8.30 (t, J = 5.6 Hz, 1H), 8.19 (s, 1H), 7.85 (t, J = 8.8 Hz, 1H), 7.76 (dd, J = 14 Hz, J = 2 Hz, 1 H), 7.64 (d, J = 8.8 Hz, 2H), 7.49 (d, J = 8.8 Hz, 1H), 6.97 (d, J = 8.4 Hz, 2H), 4.78 (m, 1H), 4.19 (t, J = 9.2 Hz, 1H), 3.80 (m, 4 H), 3.45 (t, J = 5.6 Hz, 2H), 1.85 (s, 3H). ^{13}C NMR (DMSO-d_6): δ 170.00, 158.10, 154.37, 153.99,

151.92, 138.30, 127.09, 126.60, 125.00, 124.06, 123.61, 123.19, 114.14, 114.01, 106.22, 71.79, 55.06, 47.19, 41.34, 22.42. HRMS: Q-TOF: 447.1443.

(S)-N-((3-(4-(4-(4-chlorophenyl)-1H-pyrazol-1-yl)-3-fluorophenyl)-2-oxooxazolidin-5-yl)methyl)acetamide (10d)

Following general procedure B, the product **10d** was isolated as a white solid (224 mg, yield of 72.2%), mp 237.5-238.2°C, HPLC: 98.2%. ^{1}H- NMR(DMSO-d_6): δ 8.69 (s, 1H), 8.31 (s, 2H), 7.84 (t, J = 8.8 Hz, 1H), 7.77 (m, 3H), 7.48 (m, 3H), 4.78 (m, 1H), 4.19 (t, J = 9.2 Hz, 1H), 3.80 (t, J = 6.8 Hz, 1H), 3.45 (t, J = 5.2 Hz, 2H), 1.85 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 170.00, 154.48, 153.98, 152.02, 138.60, 130.94, 130.58, 128.81, 128.27, 127.04, 125.16, 123.01, 122.75, 114.00, 106.20, 71.80, 47.20, 41.35, 22.42. HRMS: Q-TOF: 451.0954.

(S)-6-(1-(4-(5-(acetamidomethyl)-2-oxooxazolidin-3-yl)-2-fluorophenyl)-1H-pyrazol-4-yl)nicotinic acid (10e)

Following general procedure B, the product **10e** was isolated as a white solid (171 mg, yield of 78.1%), mp 279.6-280.2°C, HPLC: 98.5%. ^{1}H NMR(DMSO-d_6): δ 13.34 (s, 1H), 9.05 (d, J = 0.8 Hz, 1H), 8.88 (d, J = 1.6 Hz, 1H), 8.46 (s, 1H), 8.29 (m, 2H), 7.96 (d, J = 8.4 Hz, 1H), 7.88 (t, J = 8.8 Hz, 1H), 7.78 (dd, J = 13.6 Hz, J = 1.6 Hz, 1H), 7.51 (d, J = 9.2 Hz, 1H), 4.79 (m, 1H), 4.19 (t, J = 9.2 Hz, 1H), 3.81 (m, 1H), 3.45 (t, J = 5.2 Hz, 2H), 1.85 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 170.01, 166.15, 154.44, 153.96, 152.09, 150.53, 139.73, 138.82, 137.76, 130.56, 125.20, 123.95, 122.68, 119.46, 114.00, 106.18, 105.92, 71.80, 47.20, 41.35, 22.42; HRMS: Q-TOF: 462.1193.

(S)-N-((3-(3-fluoro-4-(4-(pyridin-2-yl)-1H-pyrazol-1-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)acetamide (10f)

Following general procedure B, the product **10f** was isolated as a white solid (296 mg, yield of

73.5%), mp 204.3°C, HPLC: 99.8%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.61 (d, J = 4 Hz, 1H), 8.52 (d, J = 6.8 Hz, 1H), 8.22 (s, 1H), 7.94 (t, J = 8.8 Hz, 1H), 7.77-7.69 (m, 2H), 7.55 (d, J = 8 Hz, 1H), 7.27-7.26 (m, 1H), 7.18-7.15 (m, 1H), 6.06 (t, J = 6 Hz, 1H), 4.86-4.80 (m, 1H), 4.10 (t, J = 9.2 Hz, 1H), 3.86-3.82 (m, 1H), 3.72-3.64 (m, 2H), 2.04 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 170.51, 154.47, 152.94, 151.26, 149.94, 139.70, 139.15, 137.43, 129.96, 125.61, 125.19, 123.42, 122.19, 120.38, 114.52, 106.68, 72.29, 47.70, 41.84, 22.91; HRMS: Q-TOF: 396.1470.

(E)-3-(dimethylamino)-2-(pyridin-3-yl)acrylonitrile (12)

The solution of 3-pyridylacetonitrile (**11**) (1.0 g, 8.46 mmol) in DMF-DMA (1.7 mL, 12.7 mmol) was reacted under microwave condition (40W, 100°C, CEM Discover SP) for 30 min. Then, the excess DMF-DMA was removed under reduced pressure. Purification by silica gel chromatography gave the title compound as yellow solid (1.06 g, yield of 72 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.11 (s, 1H), 8.52 (d, J = 4.4 Hz, 1H), 8.15 (t, J = 2.4 Hz, 1H), 7.97 (s, 1H), 7.81 (t, J = 8.8 Hz, 1H), 3.32 (s, 6H).

4-(pyridin-3-yl)-1H-pyrazol-3-amine (13)

Hydrazine hydrate (0.67 mL, 13.9 mmol) was added dropwise to a stirring solution of compound **12** (1.2 g, 6.9 mmol) in ethanol (20 mL). The mixture was heated to reflux for about 16 hours and concentrated to dryness. The residue was triturated with petroleum ether (15 mL). The suspension solid was collected on a filter and dried to yield the desired product **13** (0.51 g, yield of 46 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.56 (br s, 1H), 9.05 (s, 1H), 8.39 (d, J = 4.4 Hz, 1H), 8.08 (t, J = 2.4 Hz, 1H), 7.85 (br s, 1 H), 7.81 (t, J = 8.8 Hz, 1H), 5.89 (br s, 2H).

1-(2-fluoro-4-nitrophenyl)-4-(pyridin-3-yl)-1H-pyrazol-3(5)-amine (14)

To a stirred solution of compound **13** (700 mg, 4.37 mmol) in DMF (8 mL) was added K_2CO_3 (904 mg, 6.56 mmol) in one portion at room temperature. After 3 min stirring 1, 2-difluoro-4-nitrobenzene

(483 μL, 4.37 mmol) was added to the mixture. The reaction mixture was allowed to stir at room temperature for about 20 hours. When coupling was completed as indicated by TLC, water (25 mL) was added into the slurry with stirring. Then, the resulting slurry was stirred for 10 min, and filtered. The filtered cake was washed with water (5 mL × 3) to remove base substance and dried to give mixture **14** (902 mg, yield of 69 %). The ^{1}H NMR data was shown in Figure 2.

3-(1-(2-fluoro-4-nitrophenyl)-1H-pyrazol-4-yl)pyridine (15)

Reactor was charged with DMF (15 mL) and heated to 50 °C, and 3-methyl-1-nitroso-oxybutane (765 μL, 5.68 mmol) was added in one portion. The solid **14** (850 mg, 2.84 mmol) was then added to the mixture in batches to stabilize the nitrogen release rate. The reaction mixture was slowly cooled to 55 °C and stirred for 2 h. When TLC analysis indicated complete conversion, the water (45 mL) was charged with stirring. The resulting slurry was cooled to 25 °C, allowed to stand for more than 30 min, and filtered. The filtered cake was washed with water (3 mL × 3) and dried to give the title compound (662 mg, yield of 81.9 %) as a brown solid.

Benzyl (3-fluoro-4-(4-(pyridin-3-yl)-1H-pyrazol-1-yl)phenyl)carbamate (17)

To a stirring solution of compound **15** (640 mg, 2.25 mmol) in EtOH / H_2O (v/v = 20/10 mL) was added NH_4Cl (481.4 mg, 9.01 mmol). The solution was heated to 60 °C and Fe powder (378.6 mg, 6.76 mmol) was added over a period of 15 min. The reaction mixture was stirred for 4 hours at 60 °C. Solid was filtered off and washed well with ethanol (20 mL). The combined organic layer was concentrated and the residue was partitioned between DCM and H_2O (v/v = 20/10 mL). The organic layer was dried and concentrated to afford the desired compound **16** that was carried on to the next reaction without further purification. The amine compound **16** was dissolved in DCM, and the mixture was added K_2CO_3 (325.7 mg, 2.36 mmol). After stirred for additional 10 min at 0 °C, benzyl chloroformate (438 mg, 2.56

mmol) was added dropwise to the mixture over a 2 min period. The reaction mixture was stirred overnight at room temperature, and washed with water and brine successively. The organic layer was dried and concentrated. The residue was recrystalled from ethyl acetate to afford compound **17** (646.7 mg, yield of 74 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.17 (s, 1H), 8.62 (d, J = 4.4 Hz, 1H), 8.49 (d, J = 4.8 Hz, 1H), 8.21 (s, 1H), 8.11 (d, J = 8.0 Hz, 1H), 7.85 (t, J = 8.8 Hz, 1H), 7.71-7.67 (m, 1H), 7.38-7.25 (m, 6H), 6.92 (s, 1H), 6.81 (d, J = 2.2 Hz, 1H), 5.20 (s, 2H).

(S)-N-((3-(3-fluoro-4-(4-(pyridin-3-yl)-1H-pyrazol-1-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)acetamide (10g) (General procedure C for the oxazolidinone ring construction)

Compound **17** (600 mg, 1.54 mmol, 1.0 eq) and *(S)*-1-acetamido-3-chloropropan-2-yl acetate (598 mg, 3.08 mmol, 2.0 eq) were dissolved in THF (6 mL) under nitrogen atmosphere at -5°C. Then MeOH (137 μL, 3.08 mmol, 2.0 eq) was added in the reaction mixture in one portion. After stirred for additional 10 min, *t*-BuOLi (370 mg, 4.62 mmol, 3.0 eq) was added into the stirring solution in one portion. The mixture was allowed to stir at room temperature overnight. The resulting slurry was added acetic acid (176 μL, 3.08 mmol, 2.0 eq) in one portion and stirred for additional 5 min. Then, the mixture was evaporated under reduced pressure. The residue was resuspended with water and stirred for 2 hours. The slurry was filtered and the solid was washed well with water (5 mL× 2) to remove the basic substance. Filtered cake was collected and dried to give target compound **11g** (340 mg, yield of 56 %), mp 198.7-199.8°C, HPLC: 99.5%. ^{1}H NMR (DMSO-d_6): δ 8.98 (d, J = 1.8 Hz, 1H), 8.76 (d, J = 1.8 Hz, 1H), 8.45 (dd, J = 4.8 Hz, 1.6 Hz, 1H), 8.36 (s, 1H), 8.25 (t, J = 5.8 Hz, 1H), 8.14 - 8.08 (m, 1H), 7.85 (s, 1H), 7.77 (dd, J = 13.8 Hz, 2.4 Hz, 1H), 7.53 - 7.46 (m, 1H), 7.43 (dd, J = 8.0 Hz, 4.8 Hz, 1H), 4.78 (dt, J = 11.4 Hz, 5.2 Hz, 1H), 4.19 (s, 1H), 3.81 (dd, J = 9.2 Hz, 6.6 Hz, 1H), 3.44 (t, J = 5.6 Hz, 2H), 1.84 (s, 3H). ^{13}C NMR (DMSO-d_6): δ 175.05, 153.33, 152.88, 152.15, 151.42, 138.95, 138.91, 138.15,

120.03, 125.45, 122.34, 121.27, 119.85, 113.82, 106.22, 105.76, 71.81, 47.44, 41.69, 22.23; HRMS: Q-TOF: 396.1472.

General Procedure D for the Synthesis of 3-aromatic substituted pyrazole18a-c

To a stirring solution of corresponding **7** (1.0 eq) in EtOH was added hydrazine hydrate (1.2 eq) and acetate acid (1.0 eq) in one portion at room temperature. Then, the reaction mixture was refluxed for about 9 h followed by evaporating the solvent under reduced pressure to give the corresponding products **18**. The target solid may be used directly in the next transformation without further purification.

2-(1H-pyrazol-3-yl)pyridine (18a)

According to the general procedure for synthesis of corresponding **18**, compound **7e** (2.0 g, 11.3 mmol) was reacted with hydrazine hydrate (1.1 mL, 22.6 mmol) to give title compound **18a** (1.4 g, yield of 88%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.52 (d, J = 4.8 Hz, 1H), 7.75 (t, J = 8.0 Hz, 1H), 7.34 (d, J = 7.8 Hz, 1H), 7.57 (d, J = 2.4 Hz, 1H), 7.04 (t, J = 5.6 Hz, 1 H), 6.75 (s, 1H).

3-(1H-pyrazol-3-yl)pyridine (18b)

According to the general procedure of synthesis of corresponding **18**, compound **7a** (1.6 g, 9.1 mmol) was reacted with hydrazine hydrate (0.88 mL, 18.2 mmol) to give title compound **18b** (1.1 g, yield of 84%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.10 - 9.04 (m, 1H), 8.56 (dd, J = 4.8 Hz, 1.0 Hz, 1H), 8.10 (d, J = 7.8 Hz, 1H), 7.66 (d, J = 2.4 Hz, 1H), 7.38 - 7.31 (m, 1H), 6.67 (d, J = 2.4 Hz, 1H).

4-(1H-pyrazol-3-yl)pyridine (18c)

According to the general procedure of synthesis of corresponding **18**, compound **7h** (2.2 g, 12.5 mmol) was reacted with hydrazine hydrate (1.2 mL, 25 mmol) to give title compound **18c** (1.5 g, yield of 83%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.63 (dd, J = 4.6 Hz, 1.6 Hz, 2H), 7.71 (dd, J = 4.6 Hz, 1.6 Hz,

2H), 7.67 (d, *J* = 2.4 Hz, 1H), 6.74 (d, *J* = 2.4 Hz, 1H).

General Procedure for the Synthesis of 19a-c

To a stirring solution of corresponding **18** (1.0 eq) and K_2CO_3 (1.5 eq) in DMSO was added 3, 4-difluoronitrobenzene (1.2 eq) dropwise. Then, the reaction mixture was stirred overnight at room temperature. After completion of reaction, the mixture was poured into water and the resultant mixture was filtered under reduced pressure. The filtered cake was washed with water to remove the excess basic substance and DMSO. Then the solid was dried to constant in an oven at 60 °C to give compound **19**. The target yellow solid was used directly in the next step without further purification.

2-(1-(2-fluoro-4-nitrophenyl)-1H-pyrazol-3-yl)pyridine (19a)

According to the general procedure of synthesis of corresponding **19**, compound **18a** (1.5 g, 10.3 mmol) was reacted with 3, 4-difluoronitrobenzene (1.38 mL, 12.4 mmol) in the presence of K_2CO_3 (2.1 g, 15.5 mmol) to give target compound **19a** (2.1 g, yield of 70%).

3-(1-(2-fluoro-4-nitrophenyl)-1H-pyrazol-3-yl)pyridine (19b)

According to the general procedure of synthesis of corresponding **19**, compound **18b** (2.5 g, 17.2 mmol) was reacted with 3, 4-difluoronitrobenzene (2.3 mL, 20.7 mmol) in the presence of K_2CO_3 (3.6 g 25.8 mmol) to give target compound **19b** (3.1 g, yield of 64%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.14 (s, 1H), 8.64 (d, *J* = 4.4 Hz, 1H), 8.40 (t, *J* = 8.4 Hz, 1H), 8.28 (t, *J* = 2.6 Hz, 1H), 8.25 - 8.14 (m, 3H), 7.40 (dd, *J* = 8.0 Hz, 5.0 Hz, 1H), 6.93 (d, *J* = 2.6 Hz, 1H).

4-(1-(2-fluoro-4-nitrophenyl)-1H-pyrazol-3-yl)pyridine (19c)

According to the general procedure of synthesis of compound **19**, compound **18c** (2.3 g, 15.8 mmol) was reacted with 3, 4-difluoronitrobenzene (2.1 mL, 19 mmol) in the presence of K_2CO_3 (3.3 g, 23.7 mmol) to give target compound **19c** (3.7 g, yield of 83%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.72 (d, *J* =

5.0 Hz, 2H), 8.39 (t, *J* = 8.4 Hz, 1H), 8.28 (t, *J* = 2.6 Hz, 1H), 8.19 (dd, *J* = 16.4 Hz, 5.6 Hz, 2H), 7.80 (d, *J* = 5.8 Hz, 2H), 6.96 (d, *J* = 2.4 Hz, 1H).

General procedure for the Synthesis of compound (21a-c)

To a stirring solution of compound **19a-c** (1.0 eq) in EtOH / H_2O (v/v = 2/1) was added NH_4Cl (4.0 eq). The solution was heated to 60 °C and Fe powder (3.0 eq) was added over a period of 15 min. The reaction mixture was stirred for 4 hours at 60 °C. Solid was filtered off and washed well with ethanol. The combined organic layer was concentrated and the residue was partitioned between DCM and H_2O. The organic layer was dried and concentrated to afford the desired compound **20**, which was carried on to the next reaction without further purification. The compound **20** was dissolved in DCM, and the mixture was added K_2CO_3 (1.5 eq). After stirred for additional 10 min at 0 °C, benzyl chloroformate (1.5 eq) was added dropwise to the mixture over a 2 min period. The reaction mixture was stirred overnight at room temperature, and washed with water and brine successively. The organic layer was dried and concentrated. The residue was recrystallized from ethyl acetate to afford compound **21** (yield of 66-78%).

Benzyl (3-fluoro-4-(3-(pyridin-2-yl)-1H-pyrazol-1-yl)phenyl)carbamate (21a)

According to the above noted general procedure for synthesis of amine compounds, nitro compound **19a** was reduced through Fe powder/NH_4Cl complex to give the corresponding amine **20a** which was protected with Cbz-Cl in DCM to furnish title compound **21a**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.51 (d, *J* = 6.8 Hz, 1H), 8.09 (t, *J* = 2.4 Hz, 1H), 7.94-7.82 (m, 2H), 7.71 (d, *J* = 13.2 Hz, 1H), 7.57 (d, *J* = 8 Hz, 1H), 7.40 - 7.29 (m, 6H), 7.16 (d, *J* = 8.0 Hz, 1H), 7.03 (s, 1H), 6.88 (d, *J* = 2.4 Hz, 1H), 5.27 (s, 2H).

Benzyl (3-fluoro-4-(3-(pyridin-3-yl)-1H-pyrazol-1-yl)phenyl)carbamate (21b)

According to the above noted general procedure for synthesis of amine compounds, nitro compound **19b** was reduced through Fe powder/NH_4Cl complex to give the corresponding amine **20b** which was protected with Cbz-Cl in DCM to furnish title compound **21b**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.11 (s, 1H), 8.58 (d, J = 4.4 Hz, 1H), 8.19 (d, J = 8.0 Hz, 1H), 8.02 (t, J = 2.4 Hz, 1H), 7.90 (t, J = 8.8 Hz, 1H), 7.65 (d, J = 13.2 Hz, 1H), 7.45 - 7.31 (m, 6H), 7.10 (d, J = 8.0 Hz, 1H), 6.92 (s, 1H), 6.81 (d, J = 2.4 Hz, 1H), 5.23 (s, 2H).

Benzyl (3-fluoro-4-(3-(pyridin-4-yl)-1H-pyrazol-1-yl)phenyl)carbamate (21c)

According to the above noted general procedure for synthesis of amine compounds, nitro compound **19c** was reduced through Fe powder/NH_4Cl complex to give the corresponding amine **20c** which was protected with Cbz-Cl in DCM to furnish title compound **21c**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.59 (d, J = 5.6 Hz, 2H), 8.49 (d, J = 4.0 Hz, 1H), 7.81 (d, J = 6.0 Hz, 2H), 7.60 (d, J = 12.6 Hz, 1H), 7.39 - 7.22 (m, 6H), 7.06 (d, J = 8.4 Hz, 1H), 6.87 (s, 1H), 6.74 (d, J = 2.2 Hz, 1H), 5.25 (s, 2H).

(S)-N-((3-(3-fluoro-4-(3-(pyridin-2-yl)-1H-pyrazol-1-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)acetamide (22a)

According to general procedure C of oxazolidinone ring construction, using compound **21a** (500 mg) as starting material, compound **22a** was obtained as white solid (264 mg, yield of 41%) through chromatographic purification method. ^{1}H NMR (400 MHz, DMSO) δ 8.64 (d, J = 4.4 Hz, 1H), 8.35 – 8.22 (m, 2H), 8.03 (t, J = 11.8 Hz, 1H), 7.87 (dt, J = 7.8 Hz, 5.4 Hz, 2H), 7.76 (dt, J = 18.6 Hz, 9.4 Hz, 1H), 7.58 - 7.46 (m, 1H), 7.46 - 7.30 (m, 1H), 7.11 (d, J = 2.4 Hz, 1H), 4.89 - 4.72 (m, 1H), 4.20 (t, J = 9.0 Hz, 1H), 3.82 (dd, J = 9.0 Hz, 6.6 Hz, 1H), 3.46 (t, J = 5.4 Hz, 2H), 1.86 (s, 3H).^{13}C NMR (DMSO-d_6) δ 170.00, 154.64, 153.99, 152.52, 152.19, 151.00, 149.40, 138.60, 136.95, 133.12, 125.25, 123.70, 119.75, 114.07, 106.24, 105.98, 71.81, 47.24, 41.36, 22.42. HRMS: Q-TOF: 396.1469.

(S)-N-((3-(3-fluoro-4-(3-(pyridin-3-yl)-1H-pyrazol-1-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)acetamide (22b)

According to general procedure C of oxazolidinone ring construction, using compound **21b** (1.0 g) as starting material, compound **22b** was obtained as white solid (641 mg, yield of 34%) through chromatographic purification method. ^{1}H NMR (400 MHz, DMSO) δ 9.12 (s, 1H), 8.57 (d, J = 3.6 Hz, 1H), 8.35 - 8.24 (m, 3H), 7.92 (t, J = 8.8 Hz, 1H), 7.76 (d, J = 13.8 Hz, 1H), 7.49 (t, J = 6.4 Hz, 2H), 7.17 (d, J = 2.4 Hz, 1H), 4.78 (dt, J = 11.2 Hz, 5.4 Hz, 1H), 4.19 (t, J = 9.0 Hz, 1H), 3.86 – 3.77 (m, 1H), 3.45 (t, J = 5.4 Hz, 2H), 1.82 (s, 3H). ^{13}C NMR (DMSO-d_6) δ 170.01, 154.56, 153.99, 152.11, 149.10, 146.73, 138.57, 133.32, 132.69, 128.23, 125.22, 123.87, 122.96, 114.08, 106.11, 105.39, 71.80, 47.24, 41.36, 22.42. HRMS: Q-TOF: 396.1475.

(S)-N-((3-(3-fluoro-4-(3-(pyridin-4-yl)-1H-pyrazol-1-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)acetamide (22c)

According to general procedure C of oxazolidinone ring construction, using compound **21c** (1.0 g) as starting material, compound **22c** was obtained as white solid (677 mg, yield of 42%) through chromatographic purification method. ^{1}H NMR (400 MHz, DMSO) δ 8.64 (d, J = 6.0 Hz, 2H), 8.32 (s, 1H), 8.27 (t, J = 5.6 Hz, 1H), 7.92 (d, J = 8.8 Hz, 1H), 7.87 (d, J = 6.2 Hz, 2H), 7.77 (dd, J = 14.0 Hz, 2.2 Hz, 1H), 7.51 (d, J = 9.0 Hz, 1H), 7.23 (d, J = 2.4 Hz, 1H), 4.79 (td, J = 11.4 Hz, 5.4 Hz, 1H), 4.19 (t, J = 9.0 Hz, 1H), 3.81 (dd, J = 9.0 Hz, 6.6 Hz, 1H), 3.45 (t, J = 5.4 Hz, 2H), 1.85 (s, 3H). ^{13}C NMR (DMSO-d_6) δ 170.03, 154.69, 153.98, 152.23, 150.23, 149.50, 139.48, 138.81, 133.62, 125.36, 122.86, 119.82, 114.07, 106.10, 71.82, 47.24, 41.35, 22.41. HRMS: Q-TOF: 396.1471.

6-(1, 3-dioxopropan-2-yl) nicotinamide (23a)

To a stirred solution of 6-(1, 3-dioxopropan-2-yl) nicotinic acid (100 mg, 0.518 mmol) in DMF (4

mL) was added HOBT (101 mg, 0.745mmol), EDCI (143 mg, 0.745 mmol), NH_4Cl (166 mg, 3.11 mmol) and 4-methylmorpholine (81 μL, 0.745 mmol) at room temperature. The reaction mixture was heated to 60 °C and stirred overnight. After completion determined by TLC the mixture was poured into water (10 mL) and extracted with DCM (5 mL × 3). The organic layer was dried and concentrated. The residue was purified by chromatography on silica gel to afford compound **23a** (46 mg, yield of 47%). ^{1}H NMR (400 MHz, DMSO) δ 15.98 (s, 1H), 9.32 (s, 2H), 8.91 - 8.80 (m, 2H), 8.55 (d, J = 9.2 Hz, 1H), 8.25 (s, 1H), 7.74 (s, 1H).

6-(1, 3-dioxopropan-2-yl)-N-methylnicotinamide (23b)

To a stirred solution of 6-(1, 3-dioxopropan-2-yl) nicotinic acid (100 mg, 0.518 mmol) in DMF (4 mL) was added HOBT (101 mg, 0.745 mmol), EDCI (143 mg, 0.745 mmol), methylamine hydrochloride (210 mg, 3.11 mmol) and 4-methylmorpholine (425 μL, 7.44 mmol) at room temperature. The reaction mixture was heated to 60 °C and stirred overnight. After completion determined by TLC the mixture was poured into water (10 mL) and extracted with DCM (5 mL× 3). The organic layer was dried and concentrated. The residue was purified by chromatography on silica gel to afford compound **23b** (69 mg, yield of 65%). ^{1}H NMR (400 MHz, DMSO) δ 15.96 (s, 1H), 9.32 (s, 2H), 8.87 (d, J = 9.2 Hz, 2H), 8.75 (s, 1H), 8.50 (d, J = 9.2 Hz, 1H), 2.81 (d, J = 4.2 Hz, 3H).

2-(5-(morpholine-4-carbonyl) pyridin-2-yl)malonaldehyde (23c)

To a stirred solution of 6-(1, 3-dioxopropan-2-yl) nicotinic acid (200 mg, 1.04 mmol) in DMF (4 mL) was added HOBT (203 mg, 1.5 mmol), EDCI (288 mg, 1.5 mmol), morpholine (541 μL, 6.21 mmol) and 4-methylmorpholine (507 μL, 4.62 mmol) at room temperature. The reaction mixture was heated to 60 °C and stirred overnight. After completion determined by TLC the mixture was poured into water (10 mL) and extracted with DCM (5 mL × 3). The organic layer was dried and concentrated. The

residue was purified by chromatography on silica gel to afford compound **23c** (166 mg, yield of 61%). ^{1}H NMR (400 MHz, DMSO) δ 16.01 (s, 1H), 9.31 (s, 2H), 8.87 (d, J = 8.8 Hz, 1H), 8.57 (s, 1H), 8.22 (d, J = 8.8 Hz, 1H), 3.61 (br s, 4H), 3.52 (br s, 4H).

Methyl 6-(1, 3-dioxopropan-2-yl)nicotinate (23d)

To a stirred solution of 6-(1, 3-dioxopropan-2-yl) nicotinic acid (150 mg, 0.78 mmol) in DMF (4 mL) was added K_2CO_3 (160 mg, 1.16 mmol) followed by dropping MeI (73 μL, 1.16 mmol) into the mixture at room temperature. The reaction mixture was heated to 60 °C and stirred overnight. After completion determined by TLC the mixture was poured into water (10 mL) and extracted with DCM (5 mL × 3). The organic layer was dried and concentrated. The residue was purified by chromatography on silica gel to afford compound **23d** (84 mg, yield of 52 %). ^{1}H NMR (400 MHz, DMSO) δ 15.96 (s, 1H), 9.27 (s, 2H), 8.98 (s, 1H), 8.89 (d, J = 9.2 Hz, 1H), 8.51 (d, J = 9.0 Hz, 1H), 3.89 (s, 3H).

(S)-6-(1-(4-(5-(acetamidomethyl)-2-oxooxazolidin-3-yl)-2-fluorophenyl)-1H-pyrazol-4-yl)nicotinamide (24a)

Following general procedure B, starting from compound **23a**, product was collected by filtration to afford desired compound **24a** as a pale yellow solid (68 mg, yield of 75%). ^{1}H NMR (400 MHz, DMSO) δ 9.03 (s, 1H), 8.88 (s, 1H), 8.47 (s, 1H), 8.29 (d, J = 6.8 Hz, 2H), 8.18 (s, 1H), 7.97 (d, J = 8.4 Hz, 1H), 7.88 (t, J = 9.0 Hz, 1H), 7.79 (d, J = 14.0 Hz, 1H), 7.59 (s, 1H), 7.51 (d, J = 8.8 Hz, 1H), 4.84 - 4.71 (m, 1H), 4.20 (t, J = 8.8 Hz, 1H), 3.88 - 3.76 (m, 1H), 3.45 (t, J = 5.4 Hz, 2H), 1.85 (s, 3H). ^{13}C NMR (100 MHz, DMSO) δ 170.03, 165.15, 154.59, 153.97, 152.13, 146.34, 139.99, 139.00, 138.45, 131.28, 127.83, 125.27, 122.46, 120.53, 114.11, 106.24, 105.98, 71.80, 47.24, 41.35, 22.42. HRMS: Q-TOF: 439.1525.

(S)-6-(1-(4-(5-(acetamidomethyl)-2-oxooxazolidin-3-yl)-2-fluorophenyl)-1H-pyrazol-4-yl)-N-methylni

cotinamide (24b)

Following general procedure B, starting from compound **23b**, product was collected by filtration to afford desired compound **24b** as a pale yellow solid (96 mg, yield of 73%). ^{1}H NMR (400 MHz, DMSO) δ 8.98 (s, 1H), 8.82 (s, 1H), 8.59 (d, J = 4.2 Hz, 1H), 8.43 (s, 1H), 8.26 (t, J = 5.4 Hz, 1H), 8.20 (d, J = 8.4 Hz, 1H), 7.89 (dd, J = 21.4 Hz, 8.6 Hz, 2H), 7.77 (d, J = 13.8 Hz, 1H), 7.50 (d, J = 9.0 Hz, 1H), 4.83 - 4.74 (m, 1H), 4.19 (t, J = 9.0 Hz, 1H), 3.85 - 3.76 (m, 1H), 3.45 (t, J = 5.2 Hz, 2H), 2.82 (d, J = 3.8 Hz, 3H), 1.85 (s, 3H). ^{13}C NMR (DMSO-d_6) δ 170.02, 164.92, 154.57, 152.85, 152.12, 148.46, 139.57, 138.85, 135.58, 130.27, 127.43, 125.21, 124.05, 122.74, 119.21, 114.06, 106.24, 71.81, 47.22, 41.36, 26.12, 22.41. HRMS: Q-TOF: 453.1687.

(S)-N-((3-(3-fluoro-4-(4-(5-(morpholine-4-carbonyl)pyridin-2-yl)-1H-pyrazol-1-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)acetamide (24c)

Following general procedure B, starting from compound **23c**, product was collected by filtration to afford desired compound **24c** as a white solid (196 mg, yield of 68%). ^{1}H NMR (400 MHz, DMSO) δ 8.81 (s, 1H), 8.62 (s, 1H), 8.41 (s, 1H), 8.26 (t, J = 5.6 Hz, 1H), 7.93 - 7.83 (m, 3H), 7.77 (d, J = 13.8 Hz, 1H), 7.50 (d, J = 9.2 Hz, 1H), 4.83 - 4.75 (m, 1H), 4.19 (t, J = 9.0 Hz, 1H), 3.86 - 3.78 (m, 1H), 3.63 (s, 6H), 3.45 (t, J = 5.2 Hz, 4H), 1.85 (s, 3H). ^{13}C NMR (DMSO-d_6) δ 170.00, 166.91, 154.56, 153.98, 152.10, 151.72, 147.95, 139.45, 138.83, 135.97, 130.10, 128.89, 125.18, 124.08, 122.86, 119.31, 114.07, 106.25, 105.99, 71.81, 66.02, 47.23, 41.36, 22.41. HRMS: Q-TOF: 531.1766.

(S)-methyl6-(1-(4-(5-(acetamidomethyl)-2-oxooxazolidin-3-yl)-2-fluorophenyl)-1H-pyrazol-4-yl)nicotinate (24d)

Following general procedure B, starting from compound **23d**, product was collected by filtration to afford desired compound **24d** as a pale yellow solid (114 mg, yield of 70%). ^{1}H NMR (400 MHz,

DMSO) δ 9.07 (s, 1H), 8.89 (s, 1H), 8.46 (s, 1H), 8.28 (dd, J = 16.8, 7.2 Hz, 2H), 7.98 (d, J = 8.4 Hz, 1H), 7.88 (t, J = 8.8 Hz, 1H), 7.78 (d, J = 13.8 Hz, 1H), 7.51 (d, J = 9.2 Hz, 1H), 4.82 - 4.74 (m, 1H), 4.19 (t, J = 8.8 Hz, 1H), 3.90 (s, 3H), 3.86 - 3.77 (m, 1H), 3.45 (t, J = 5.4 Hz, 2H), 1.85 (s, 3H). ^{13}C NMR (DMSO-d_6) δ 170.02, 165.09, 154.69, 153.97, 152.14, 150.28, 139.78, 138.93, 137.63, 130.72, 125.23, 123.79, 123.00, 122.73, 119.58, 114.05, 106.22, 71.80, 52.25, 47.22, 41.36, 22.41. HRMS: Q-TOF: 476.1343.

2-(quinolin-2-yl)propane-1,3-diol (25)

The solution of 2-quinaldine (2.0 g, 13.9 mmol) and formalin (4.1 mL, 55.5 mmol) was stirred at 130 °C for 7 hours. The excess formalin was evaporated and the residue was purified by chromatography on silica gel to afford compound **25** (1.27 g, yield of 45%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (d, J = 8.4 Hz, 1H), 8.03 (d, J = 8.4 Hz, 1H), 7.81 (d, J = 8.0 Hz, 1H), 7.72 (t, J = 7.8 Hz, 1H), 7.54 (t, J = 7.6 Hz, 1H), 7.38 (d, J = 8.4 Hz, 1H), 4.28 (dd, J = 11.2 Hz, 4.8 Hz, 2H), 4.21 - 4.12 (m, 2H), 3.98 (s, 2H), 3.24 (p, J = 4.4 Hz, 1H).

2-(quinolin-2-yl)malonaldehyde (26)

To a stirred solution of compound **25** (200 mg, 0.98 mmol) in DMSO (10 mL) and DCM (2 mL) was added Et_3N (800 mg, 7.88 mmol) in one portion. Pyridine sulfur trioxide (1.1 g, 6.9 mmol) was added to the stirring solution at 0 °C. After 2 hours stirring, the mixture was poured into water (30 mL), extracted with DCM (10 mL × 3). The organic layer was dried, concentrated and purified by chromatography on silica gel to afford compound **26** (78 mg, yield of 40%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.49 (s, 2H), 9.06 (d, J = 9.4 Hz, 1H), 8.29 (d, J = 9.2 Hz, 1H), 7.88-7.73 (m, 3H), 7.58 (t, J = 7.2 Hz, 1H).

(E)-3-(dimethylamino)-2-(pyrazin-2-yl)acrylaldehyde (27)

$POCl_3$ (5.0 g, 32.61 mmol) was dropped into the stirring DMF (7 mL) at 0 °C and agitated for additional 30 min followed by dropping the 2-methylpyrazine (993 μL, 10.87 mmol) at 0 °C. The mixture was gradually heated to 60 °C and stirred overnight. EtOH (25 mL) was added to the mixture followed by adding the K_2CO_3 saturated solution to buffer to pH 7-8. The mixture was extracted with DCM (20 mL × 3). The organic layer was dried, concentrated and recrystallized from toluene to afford the desired compound **27** (938 mg, yield of 49%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.20 (s, 1H), 8.77 (s, 1H), 8.57 (s, 1H), 8.38 (s, 1H), 7.06 (s, 1H), 3.26 (s, 3H), 2.66 (s, 3H).

(S)-N-((3-(3-fluoro-4-(4-(quinolin-2-yl)-1H-pyrazol-1-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)acetamide (28a)

Following general procedure B, starting from compound **26**, product was collected by filtration to afford desired compound **28a** as a white solid (94.7 mg, yield of 64.5%). ^{1}H NMR (400 MHz, DMSO) δ 8.98 (s, 1H), 8.57 (s, 1H), 8.45 (d, J = 8.3 Hz, 1H), 8.27 (t, J = 5.2 Hz, 1H), 8.08 - 8.01 (m, 2H), 7.98 (d, J = 8.1 Hz, 1H), 7.92 (t, J = 8.8 Hz, 1H), 7.79 (dd, J = 14.6 Hz, 8.2 Hz, 2H), 7.58 (t, J = 7.2 Hz, 1H), 7.52 (d, J = 8.8 Hz, 1H), 4.83 - 4.75 (m, 1H), 4.21 (t, J = 8.8 Hz, 1H), 3.91 - 3.77 (m, 1H), 3.46 (t, J = 5.0 Hz, 2H), 1.85 (s, 3H). ^{13}C NMR (DMSO-d_6) δ 170.02, 154.60, 154.00, 152.16, 150.68, 148.38, 139.95, 138.82, 130.84, 130.20, 127.96, 126.70, 126.07, 125.26, 124.26, 122.85, 119.26, 114.07, 106.26, 105.93, 71.83, 47.24, 41.36, 22.44. HRMS: Q-TOF: 446.1630.

(S)-N-((3-(3-fluoro-4-(4-(pyrazin-2-yl)-1H-pyrazol-1-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)acetamide (28b)

Following general procedure B, starting from compound **27**, product was collected by filtration to afford desired compound **28b** as a gray solid (644 mg, yield of 57.6%). ^{1}H NMR (400 MHz, DMSO) δ 9.14 (s, 1H), 8.89 (s, 1H), 8.62 (s, 1H), 8.49 (d, J = 2.4 Hz, 1H), 8.46 (s, 1H), 8.27 (t, J = 5.6 Hz, 1H),

7.88 (t, *J* = 8.8 Hz, 1H), 7.78 (dd, *J* = 14.0 Hz, 1.8 Hz, 1H), 7.51 (d, *J* = 9.2 Hz, 1H), 4.79 (m, 1H), 4.20 (t, *J* = 9.0 Hz, 1H), 3.81 (t, *J* = 6.6 Hz, 1H), 3.45 (t, *J* = 5.4 Hz, 2H), 1.85 (s, 3H). ^{13}C NMR (DMSO-d_6) δ 170.01, 154.60, 153.97, 152.15, 146.79, 144.27, 142.34, 141.74, 139.32, 130.19, 125.26, 122.74, 121.48, 114.03, 106.21, 71.81, 47.22, 41.36, 22.41. HRMS: Q-TOF: 419.1242.

(S)-N-((3-(3-fluoro-4-(4-(quinoxalin-2-yl)-1H-pyrazol-1-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)acetamide (28c)

Following general procedure B, starting from 2-(quinoxalin-2-yl)malonaldehyde, product was collected by filtration to afford desired compound **28c** as a brown solid (281 mg, yield of 63%). ^{1}H NMR (400 MHz, DMSO) *δ* 9.47 (s, 1H), 9.11 (s, 1H), 8.63 (s, 1H), 8.27 (s, 1H), 8.12 - 8.04 (m, 2H), 7.92 (t, *J* = 8.9 Hz, 1H), 7.83 (dd, *J* = 22.0 Hz, 9.8 Hz, 3H), 7.53 (d, *J* = 8.6 Hz, 1H), 4.82 - 4.76 (m, 1H), 4.21 (t, *J* = 8.8 Hz, 1H), 3.88 - 3.78 (m, 1H), 3.46 (t, *J* = 5.2 Hz, 2H), 1.85 (s, 3H). ^{13}C NMR (DMSO-d_6) δ 170.02, 154.68, 153.99, 152.22, 146.40, 144.09, 141.58, 140.66, 139.94, 131.18, 130.52, 129.12, 128.91, 128.54, 125.37, 122.59, 121.93, 114.07, 105.97, 71.82, 47.23, 41.36, 22.42. HRMS: Q-TOF: 469.1534.

(S)-N-((3-(3-fluoro-4-(4-(2-nitrophenyl)-1H-pyrazol-1-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)acetamide (28d)

Following general procedure B, starting from 2-(2-nitrophenyl)malonaldehyde, product was collected by filtration to afford desired compound **28d** as a white solid (208 mg, yield of 61%). ^{1}H NMR (400 MHz, DMSO) *δ* 8.42 (s, 1H), 8.25 (t, *J* = 5.6 Hz, 1H), 7.95 (d, *J* = 8.8 Hz, 2H), 7.85 (t, *J* = 8.8 Hz, 1H), 7.75 (m, 3H), 7.58 (m, 1H), 7.50 (d, *J* = 8.6 Hz, 1H), 4.83 - 4.72 (m, 1H), 4.19 (t, *J* = 9.0 Hz, 1H), 3.87 - 3.74 (m, 1H), 3.45 (t, *J* = 5.2 Hz, 2H), 1.84 (s, 3H). ^{13}C NMR (DMSO-d_6) δ 170.00, 154.50, 153.98, 152.05, 148.32, 139.67, 138.83, 132.67, 131.22, 130.05, 128.38, 125.17, 123.88, 122.71, 118.28,

114.08, 106.24, 71.81, 47.22, 41.35, 22.41. HRMS: Q-TOF: 440.1287.

2-(1H-pyrazol-4-yl) pyridine

Hydrazine hydrate (1.3 mL, 26.82 mmol) was added dropwise to a stirring solution of 2-pyridinylmalonaldehyde (2.0 g, 13.41 mmol) in ethanol (20 mL). The mixture was heated to reflux for about 5 hours and concentrated to dryness. The residue was recrystallized from ethanol to afford compound **16** as brown solid (1.7 g, yield of 86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.59 (d, J = 4.8 Hz, 1H), 8.15 (s, 2H), 7.69 (t, J = 7.8 Hz, 1H), 7.52 (d, J = 8.0 Hz, 1H), 7.18 - 7.11 (m, 1H).

2-(1-(4-nitrophenyl)-1H-pyrazol-4-yl) pyridine (29a)

To the stirring solution of 2-(1*H*-pyrazol-4-yl) pyridine (500 mg, 3.27 mmol) in DMSO (10 mL) was added K_2CO_3 (487 mg, 3.26 mmol) in one portion. After stirring for about 3 min, 4-fluoronitrobenzene (500 mg, 2.82 mmol) was added to the reaction mixture. The reaction mixture was allowed to stir overnight at room temperature. Upon completion of the coupling as indicated by TLC, water (30 mL) was added into the slurry with stirring. The solid was collected through filtration and washed well with ethanol. The resulting solid was dried under *vacuum* to afford the desired compound **29a** (756 mg, yield of 87%). ^{1}H NMR (400 MHz, DMSO) δ 9.34 (s, 1H), 8.60 (d, J = 4.2 Hz, 1H), 8.46 (s, 1H), 8.40 (d, J = 8.8 Hz, 2H), 8.21 (d, J = 8.8 Hz, 2H), 7.90 - 7.81 (m, 2H), 7.30 (t, J = 5.0 Hz, 1H).

2-(1-(2, 6-difluoro-4-nitrophenyl)-1H-pyrazol-4-yl) pyridine (29b)

To a stirred solution of starting material 2-(1*H*-pyrazol-4-yl) pyridine (410 mg, 2.82 mmol) in DMSO (4 mL) was added K_2CO_3 (588 mg, 4.26 mmol) in one portion at room temperature. After 3 min stirring 3, 4, 5-trifluoronitrobenzene (500 mg, 2.82 mmol) was added to the mixture. The reaction mixture was allowed to stir at room temperature overnight and then partitioned between DCM and H_2O (5 mL/10 mL). The organic layer was dried and concentrated and the residue was purified by

chromatography to afford desired compound **29b** (613 mg, yield of 72%). ^{1}H NMR (400 MHz, DMSO) δ 9.34 (s, 1H), 8.60 (d, J = 4.2 Hz, 1H), 8.46 (s, 1H), 8.40 (d, J = 8.8 Hz, 2H), 8.21 (d, J = 8.8 Hz, 1H), 7.90 - 7.81 (m, 1H), 7.30 (t, J = 5.0 Hz, 1H).

Benzyl 4-(4-(pyridin-2-yl)-1H-pyrazol-1-yl)phenylcarbamate (31a)

To a stirred solution of compound **29a** (600 mg, 2.25 mmol) in EtOH / H_2O (v/v = 20/10 mL) was added NH_4Cl (481 mg, 9.01 mmol). The solution was heated to 60 °C and Fe powder (378.5 mg, 6.76 mmol) was added over a period of 15 min. The reaction mixture was stirred for 4 hours at 60 °C. Solid was filtered off, washed well with ethanol. The combined organic layer was concentrated and the residue was partitioned between DCM and H_2O (10 mL/20 mL × 3). The organic layer was dried and concentrated to afford the desired compound **30a** that was carried on to the next reaction without further purification. The amine **30a** was dissolved in DCM (5 mL) followed by adding K_2CO_3 (465.7 mg, 3.38 mmol) to the mixture which was then allowed to stir for additional 5 min at 0 °C. Benzyl chloroformate (521 μL, 3.83 mmol) was added dropwise to the mixture over a 1 min period while the temperature was kept below 10 °C. The reaction mixture was stirred overnight at room temperature. Upon completion of the protection as indicated by TLC, the mixture was partitioned between DCM and H_2O (10 mL/20 mL × 3), the organic layer was washed with water and brine successively. The organic layer was dried and concentrated. The residue was recrystallized from EA to afford compound **31a** (681 mg, yield of 78%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.59 (d, J = 4.8 Hz, 1H), 8.46 (s, 1H), 8.15 (s, 1H), 7.72 (m, 3H), 7.56 - 7.49 (m, 3H), 7.43 - 7.35 (m, 6H), 7.15 (dd, J = 4.8 Hz, J = 6.4 Hz, 1H), 5.22 (s, 2H).

Benzyl 3,5-difluoro-4-(4-(pyridin-2-yl)-1H-pyrazol-1-yl)phenylcarbamate (31b)

Following general procedure for the synthesis of **31a** described above, starting from compound **29b**, the residue was recrystallized from EA to afford the desired compound **31b** as a white solid (477 mg,

yield of 71%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.45 (s, 1H), 8.65 (d, J = 5.4 Hz, 1H), 8.34 (s, 1H), 8.26 (s, 1H), 7.93 (d, J = 7.8 Hz, 1H), 7.59 (d, J = 12.5 Hz, 2H), 7.44 - 7.28 (m, 7H), 5.20 (s, 2H).

(S)-N-((2-oxo-3-(4-(4-(pyridin-2-yl)-1H-pyrazol-1-yl)phenyl)oxazolidin-5-yl)methyl)acetamide (32a)

Following general procedure C, starting from compound **31a**, the residue was purified by column chromatography on silica gel to afford the desired compound **32a** as a white solid (153 mg, yield of 39%). ^{1}H NMR (400 MHz, DMSO) δ 9.08 (s, 1H), 8.57 (d, J = 4.8 Hz, 1H), 8.32 (s, 1H), 8.27 (t, J = 6.0 Hz, 1H), 7.96 (d, J = 9.0 Hz, 2H), 7.83 (q, J = 7.8 Hz, 2H), 7.70 (d, J = 9.0 Hz, 2H), 7.25 (t, J = 5.4 Hz, 1H), 4.76 (td, J = 11.4 Hz, 5.4 Hz, 1H), 4.19 (t, J = 8.8 Hz, 1H), 3.81 (dd, J = 8.8 Hz, 6.6 Hz, 1H), 3.45 (t, J = 5.4 Hz, 2H), 1.85 (s, 3H). ^{13}C NMR (DMSO-d_6) δ 169.98, 154.12, 150.97, 149.40, 139.17, 136.91, 136.79, 135.08, 125.89, 124.91, 121.61, 119.80, 118.91, 118.75, 71.59, 47.27, 41.41, 22.43. HRMS: Q-TOF: 400.1386.

(S)-N-((3-(3,5-difluoro-4-(4-(pyridin-2-yl)-1H-pyrazol-1-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)acetamide (32b)

Following general procedure C, starting from compound **31b**, the residue was purified by column chromatography on silica gel to afford the desired compound **32b** as a white solid (191 mg, yield of 47%). ^{1}H NMR (400 MHz, DMSO) δ 8.68 (s, 1H), 8.57 (d, J = 4.2 Hz, 1H), 8.41 (s, 1H), 8.18 (s, 1H), 7.86 - 7.66 (m, 3H), 7.55 (d, J = 5.0 Hz, 1H), 7.26 (t, J = 5.6 Hz, 1H), 4.60 (d, J = 5.4 Hz, 1H), 3.72 (t, J = 8.4 Hz, 1H), 3.39 (t, J = 7.2 Hz, 1H), 3.31 (d, J = 7.6 Hz, 2H), 1.81 (s, 3H). ^{13}C NMR (DMSO-d_6) δ 170.03, 158.21, 155.73, 153.85, 150.71, 149.47, 139.72, 136.97, 131.90, 124.29, 121.73, 119.85, 101.73, 101.45, 71.96, 47.26, 41.31, 22.41. HRMS: Q-TOF: 414.1372.

(S)-2-(1-(4-(5-(acetamidomethyl)-2-oxooxazolidin-3-yl)-2-fluorophenyl)-1H-pyrazol-4-yl)pyridine 1-oxide (33)

To a stirred solution of compound **10f** (200 mg, 0.506 mmol) in DCM (4 mL) was added mCPBA (175 mg, 1.01 mmol) in one portion at room temperature. The reaction mixture was allowed to stir for 18 hours and poured into water. The organic layer was washed with brine, dried and concentrated. The residue was dissolved with DCM, and K_2CO_3 (279 mg, 2.02 mmol) was added to the mixture which was heated to reflux for about 4 hours. Solid was removed by filtration, and the filtrate was concentrated to afford the desired compound **33** as a white solid (148 mg, yield of 71%). ^{1}H NMR (400 MHz, DMSO) δ 9.34 (s, 1H), 8.71 (d, J = 15.6 Hz, 1H), 8.35 (d, J = 6.6 Hz, 1H), 8.29 (t, J = 5.2 Hz, 1H), 8.12 (d, J = 8.0 Hz, 1H), 7.89 (t, J = 8.8 Hz, 1H), 7.78 (d, J = 14.0 Hz, 1H), 7.51 (d, J = 9.0 Hz, 1H), 7.42 (t, J = 7.8 Hz, 1H), 7.31 (t, J = 7.0 Hz, 1H), 4.86 - 4.65 (m, 1H), 4.19 (t, J = 8.8 Hz, 1H), 3.92 - 3.74 (m, 1H), 3.45 (t, J = 5.2 Hz, 2H), 1.85 (s, 3H). ^{13}C NMR (DMSO-d_6) δ 170.01, 153.97, 152.03, 140.76, 140.41, 140.06, 138.85, 131.92, 131.84, 125.13, 123.80, 123.31, 122.65, 115.40, 114.14, 106.26, 71.80, 47.22, 41.35, 22.42. HRMS: Q-TOF: 450.0980.

(S)-N-((3-(4-(4-(6-cyanopyridin-2-yl)-1H-pyrazol-1-yl)-3-fluorophenyl)-2-oxooxazolidin-5-yl)methyl) acetamide (34)

To a stirred solution of compound **33** (100 mg, 0.243 mmol) in DCM (3 mL) was added TMSCN (64 μL, 0.486 mmol) in one portion. After stirred for 10 min, dimethylcarbamyl chloride was added to the mixture. The reaction mixture was allowed to stir at room temperature for 48 hours and poured into water. The organic layer was dried and concentrated and purified by chromatography on silica gel to afford compound **34** (47 mg, yield of 46%). ^{1}H NMR (400 MHz, DMSO) δ 8.87 (s, 1H), 8.43 (s, 1H), 8.26 (t, J = 5.6 Hz, 1H), 8.17 (d, J = 8.2 Hz, 1H), 8.08 (t, J = 7.8 Hz, 1H), 7.87 (t, J = 7.4 Hz, 2H), 7.78 (d, J = 14.0 Hz, 1H), 7.51 (d, J = 9.2 Hz, 1H), 4.85 - 4.76 (m, 1H), 4.19 (t, J = 9.0 Hz, 1H), 3.87 - 3.74 (m, 1H), 3.45 (t, J = 5.4 Hz, 2H), 1.85 (s, 3H). HRMS: Q-TOF: 443.1243.

2-(3-fluoro-1H-pyrazol-4-yl) pyridine (36a)

A suspension of compound **35** (300 mg, 1.87 mmol) in 37% HCl aqueous solution (1 mL) and H_2O (2 mL) was added a solution of $NaNO_2$ (154 mg, 2.24 mmol) in water (0.5 mL) dropwise in a period of 2 min at -5 °C. The diazotization is complete when a faint positive test for nitrous acid with starch-iodide paper persists for 30 min. A cool fluoboric acid solution (0.59 mL, 3.74 mmol) is added rapidly, with stirring, to the diazonium solution while the temperature is kept below 0 °C. The stirring is continued for 4 hours. The solid was removed by filtration, and the filtrate was extracted with DCM (5 mL). The organic layer was dried and concentrated to afford the desired compound **36a** (95 mg, yield of 31%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.81 (s, 1H), 8.64 (d, J = 4.6 Hz, 1H), 8.36 (s, 1H), 8.02 (d, J = 8.0 Hz, 1H), 7.84 (t, J = 7.6 Hz, 1H), 7.28 (m, 1H).

2-(3-chloro-1H-pyrazol-4-yl) pyridine (36b)

A suspension of compound **35** (300 mg, 1.87 mmol) in 37% HCl (1 mL) and H_2O (5 mL) was added a solution of $NaNO_2$ (154 mg, 2.24 mmol) in water (1 mL) dropwise in a period of 2 min at -5 °C. The diazotization is complete when a faint positive test for nitrous acid with starch-iodide paper persists for 30 min. While the diazotization was in process, CuCl (185 mg, 1.87 mmol) was dissolved in 37% HCl (0.5 mL) with vigorously agitation at 60 °C. After completion of diazotization, the diazotization mixture was added over a 3 min period to the vigorously stirred solution of CuCl. After stirring for an additional 4 hours, the mixture was cooled and the pH of the reaction mixture was adjusted to 7-8 by the dropwise addition of saturated K_2CO_3 solution with cooling in an ice bath. The mixture was extracted with DCM. Organic layer was dried and concentrated to afford the desired compound **36b** (85 mg, yield of 25.4%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.82 (s, 1H), 8.67 (d, J = 4.6 Hz, 1H), 8.37 (s, 1H), 8.00 (d, J = 8.0 Hz, 1H), 7.39 (t, J = 7.6 Hz, 1H), 7.23 (m, 1H).

2-(3-bromo-1H-pyrazol-4-yl) pyridine (36c)

A suspension of compound **35** (300 mg, 1.87 mmol) in 47% HBr (1 mL) and H_2O (5 mL) was added a solution of $NaNO_2$ (155 mg, 2.25 mmol) in water (1 mL) dropwise in a period of 2 min at -5 °C. The diazotization is complete when a faint positive test for nitrous acid with starch-iodide paper persists for 30 min. While the diazotization was in process, CuBr (150.6 mg, 1.05 mmol) was dissolved in 37% HBr (0.21 mL) with vigorously stirring at 60 °C. After completion of diazotization, the diazotization mixture was added over a 3 min period to the vigorously stirred solution of CuBr. After stirring for an additional 4 hours, the mixture was cooled and the pH of the reaction mixture was adjusted to 7-8 by the dropwise addition of saturated K_2CO_3 solution with cooling in an ice bath. The mixture was extracted with DCM. Organic layer was dried and concentrated to afford the desired compound **36c** (110 mg, yield of 26%). ^{1}H NMR (400 MHz, DMSO) δ 13.56 (s, 1H), 8.62 (t, J = 17.2 Hz, 1H), 8.34 (s, 1H), 8.03 - 7.75 (m, 2H), 7.34 (dd, J = 8.4 Hz, 4.0 Hz, 1H).

2-(3-fluoro-1-(2-fluoro-4-nitrophenyl)-1H-pyrazol-4-yl) pyridine (37a)

To a stirred solution of compound **36a** (90 mg, 0.552 mmol) in DMSO (2 mL) was added K_2CO_3 (114.2 mg, 0.827 mmol) in one portion at room temperature. After 3 min stirring 1, 2-difluoro-4-nitrobenzene (73 μL, 0.662 mmol) was added to the mixture. The reaction mixture was allowed to stir at room temperature for about 5 hours, partitioned between DCM and H_2O. The organic layer was dried and concentrated and the residue was purified by chromatography to afford desired compound **37a** (137 mg, yield of 82%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.77 (s, 1H), 8.68 - 8.61 (m, 1H), 8.23 (t, J = 8.2 Hz, 1H), 8.21 (d, J = 9.8 Hz, 2H), 7.99 (d, J = 8.0 Hz, 1H), 7.81 (t, J = 7.8 Hz, 1H), 7.30 - 7.24 (m, 1H).

2-(3-chloro-1-(2-fluoro-4-nitrophenyl)-1H-pyrazol-4-yl) pyridine (37b)

Compound **37b** was prepared from **36b** in the same manner as described for **37a** (122 mg, yield of 86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.75 (s, 1H), 8.69 - 8.63 (m, 1H), 8.26 (t, J = 8.2 Hz, 1H), 8.19 (d, J = 9.8 Hz, 2H), 7.97 (d, J = 8.0 Hz, 1H), 7.79 (t, J = 7.8 Hz, 1H), 7.27 (d, J = 7.0 Hz, 1H).

2-(3-bromo-1-(2-fluoro-4-nitrophenyl)-1H-pyrazol-4-yl) pyridine (37c)

Compound **37c** was prepared from **36c** in the same manner as described for **37a** (144 mg, yield of 89%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.74 (s, 1H), 8.67 (s, 1H), 8.28 (t, J = 8.2 Hz, 1H), 8.19 (d, J = 10.4 Hz, 2H), 8.04 (d, J = 8.0 Hz, 1H), 7.83 (t, J = 7.8 Hz, 1H), 7.35 - 7.28 (m, 1H).

Benzyl 3-fluoro-4-(3-fluoro-4-(pyridin-2-yl)-1H-pyrazol-1-yl) phenylcarbamate (39a)

Compound **39a** was prepared from **37a** in the same manner as described for **31a** (108 mg, yield of 67%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.63 (d, J = 4.4 Hz, 1H), 8.52 (s, 1H), 7.96 (d, J = 8.0 Hz, 1H), 7.77 (t, J = 8.4 Hz, 2H), 7.63 (d, J = 13.4 Hz, 1H), 7.38 (dd, J = 16.6 Hz, 8.4 Hz, 5H), 7.24 - 7.19 (m, 1H), 7.08 (d, J = 8.8 Hz, 1H), 6.98 (s, 1H), 5.22 (s, 2H).

Benzyl 4-(3-chloro-4-(pyridin-2-yl)-1H-pyrazol-1-yl)-3-fluorophenylcarbamate (39b)

Compound **39b** was prepared from **37b** in the same manner as described for **31a** (103 mg, yield of 71%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 (d, J = 4.2 Hz, 1H), 8.56 (s, 1H), 7.99 (d, J = 8.0 Hz, 1H), 7.82 (t, J = 8.4 Hz, 2H), 7.69 (d, J = 13.6 Hz, 1H), 7.42(dd, J = 16.4 Hz, 8.2 Hz, 5H), 7.25 - 7.20 (m, 1H), 7.09 (d, J = 8.8 Hz, 1H), 7.01 (s, 1H), 5.23 (s, 2H).

Benzyl 4-(3-bromo-4-(pyridin-2-yl)-1H-pyrazol-1-yl)-3-fluorophenylcarbamate (39c)

Compound **39c** was prepared from **37c** in the same manner as described for **31a** (102 mg, yield of 62%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.71 (d, J = 4.4 Hz, 1H), 8.65 (s, 1H), 8.07 (d, J = 8.0 Hz, 1H), 7.89 (t, J = 8.2 Hz, 2H), 7.74 (d, J = 13.4 Hz, 1H), 7.45 (dd, J = 16.4, 8.4 Hz, 5H), 7.28 - 7.23 (m, 1H), 7.17 (d, J = 8.8 Hz, 1H), 7.08 (s, 1H), 5.25 (s, 2H).

(S)-N-((3-(3-fluoro-4-(3-fluoro-4-(pyridin-2-yl)-1H-pyrazol-1-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)acetamide (40a)

Following general procedure C, starting from compound **39a**, the residue was purified by column chromatography on silica gel to afford the desired compound **40a** as a white solid (37 mg, yield of 36.5%). ^{1}H NMR (400 MHz, DMSO) δ 8.68 (s, 1H), 8.65 (d, J = 4.6 Hz, 1H), 8.26 (t, J = 5.2 Hz, 1H), 7.92 (s, 2H), 7.85 (t, J = 8.8 Hz, 1H), 7.77 (d, J = 13.8 Hz, 1H), 7.51 (d, J = 8.8 Hz, 1H), 7.35 (d, J = 5.6 Hz, 1H), 4.77 (d, J = 6.2 Hz, 1H), 4.19 (t, J = 9.2 Hz, 1H), 3.84 - 3.78 (m, 1H), 3.44 (t, J = 5.0 Hz, 2H), 1.84 (s, 3H). ^{13}C NMR (DMSO-d_6) δ 170.33, 155.17, 153.85, 152.72, 149.89, 149.66, 140.02, 138.15, 137.53, 133.71, 125.84, 123.15, 121.12, 120.45, 114.76, 107.20, 72.49, 47.61, 41.95, 22.50. HRMS: Q-TOF: 414.1305.

(S)-N-((3-(4-(3-chloro-4-(pyridin-2-yl)-1H-pyrazol-1-yl)-3-fluorophenyl)-2-oxooxazolidin-5-yl) methyl)acetamide (40b)

Following general procedure C, starting from compound **39b**, the residue was purified by column chromatography on silica gel to afford the desired compound **40b** as a white solid (20.5 mg, yield of 22.4%). ^{1}H NMR (400 MHz, DMSO) δ 8.68 (s, 1H), 8.65 (d, J = 4.6 Hz, 1H), 8.26 (t, J = 5.2 Hz, 1H), 7.92 (s, 2H), 7.85 (t, J = 8.8 Hz, 1H), 7.77 (d, J = 13.8 Hz, 1H), 7.51 (d, J = 8.8 Hz, 1H), 7.35 (d, J = 5.6 Hz, 1H), 4.77 (d, J = 6.2 Hz, 1H), 4.19 (t, J = 9.2 Hz, 1H), 3.84 - 3.78 (m, 1H), 3.44 (t, J = 5.0 Hz, 2H), 1.84 (s, 3H). ^{13}C NMR (DMSO-d_6) δ 170.08, 154.92, 153.99, 152.77, 150.21, 149.42, 139.54, 138.27, 137.10, 133.28, 125.53, 122.87, 120.47, 120.11, 114.23, 106.81, 72.35, 47.57, 41.86, 22.61. HRMS: Q-TOF: 452.1012.

(S)-N-((3-(4-(3-bromo-4-(pyridin-2-yl)-1H-pyrazol-1-yl)-3-fluorophenyl)-2-oxooxazolidin-5-yl) methyl)acetamide (40c)

Following general procedure C, starting from compound **39c**, the residue was purified by column chromatography on silica gel to afford the desired compound **40c** as a white solid (23.8 mg, yield of 26.1%). ^{1}H NMR (400 MHz, DMSO) δ 8.73 (s, 1H), 8.65 (d, J = 4.4 Hz, 1H), 8.26 (s, 1H), 7.89 (s, 2H), 7.85 (t, J = 9.0 Hz, 1H), 7.77 (d, J = 13.8 Hz, 1H), 7.50 (d, J = 8.8 Hz, 1H), 7.35 (s, 1H), 4.82 – 4.76 (m, 1H), 4.19 (t, J = 8.9 Hz, 1H), 3.85 - 3.78 (m, 1H), 3.44 (t, J = 5.6 Hz, 2H), 1.84 (s, 3H). ^{13}C NMR (DMSO-d_6) δ 170.00, 154.85, 153.97, 152.39, 149.69, 149.13, 139.26, 137.84, 137.02, 133.21, 125.46, 122.34, 120.80, 120.34, 114.04, 106.20, 71.83, 47.22, 41.34, 22.42. HRMS: Q-TOF: 474.0578, 476.0595.

ASSOCIATED CONTENT

Supporting Information

The survival rate of compound **10f** phosphate and **28b** phosphate presented in table format and the stability analysis of **10f** phosphate. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

MRSA, methicillin-resistant *Staphylococcus aureus*; MRSE, methicillin-resistant *Staphylococcus epidermidis*; VRE, vancomycin- resistant *Enterococci*; FDA, Food and Drug Administration; CbzCl, benzyl chloroformate; SAR, structure−activity relationship; hERG, human ether-a-go-go related gene;

QT, the length of time between the start of the Q wave and end of the T wave on an electrocardiogram.

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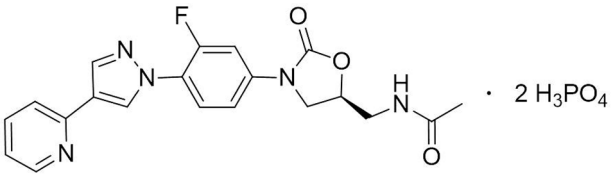
Table of Contents Graphic



MIC_{Range}: 0.25-2.0 μg/mL

CC_{50} (mammalian cells)>70μM

IC_{50} (hERG K^+ channel)>40μM

Oral bioavailability: F=99.1%

Water solubility: 47.1 mg/mL