Research Article

Synthesis, SAR and Biological Evaluation of Novel Phosphorous Containing Oxazolidinone Derivatives as Antibacterial Agents

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There is an unmet medical need to discover and develop innovative antibacterial compounds active against resistant Gram-positive bacteria. A series of novel oxazolidinone analogues having phosphorous substitution were synthesized and their antibacterial activities evaluated against a panel of resistant and susceptible Gram-positive bacteria. Potent *in vitro* antibacterial activities were exhibited by several compounds against all the organisms tested including linezolid resistant strains. To the best of our knowledge, this is the first report of phosphorous substituted phenyloxazolidinones. One of the synthesized compounds, compound **47**, has been shortlisted for further evaluation. Additionally, molecular docking studies suggest that compound **47** has additional interactions with ribosome than that of linezolid in 50S RNA.

INTRODUCTION

The emergence of bacterial resistance to existing antibiotics has become a problem worldwide.[1] Of particular concern are infections caused by multidrug-resistant gram-positive pathogens primarily methicillin-resistant Staphylococcus aureus (MRSA), Staphylococcus epidermidis (MRSE), Vancomycin-resistant Enterococcus faecium (VREF) and Glycopeptide Intermediate Resistant Staphylococcus aureus(GISA).[2-5] The alarming rate of resistance development has prompted scientists to explore novel antibacterials active against multi-drug resistant Gram-positive bacterial pathogens. The oxazolidinones, a class of synthetic antibacterial agents, are found to be active against multidrug-resistant Gram-positive bacteria that are resistant to other clinically useful antibiotics.[6-12] Linezolid **1** (Figure 1), launched by Pharmacia having the trade name Zyvox®, represents the first member of this class to receive regulatory approval. Unfortunately, within a few years, resistance against linezolid has surfaced particularly in Enterococcus faecium and in *S. aureus* strains.[13]

The discovery of linezolid and limitations associated with it has spurred intense research efforts directed towards the development of novel oxazolidinone antibacterials.[14-18] Linezolid was launched in 2000.

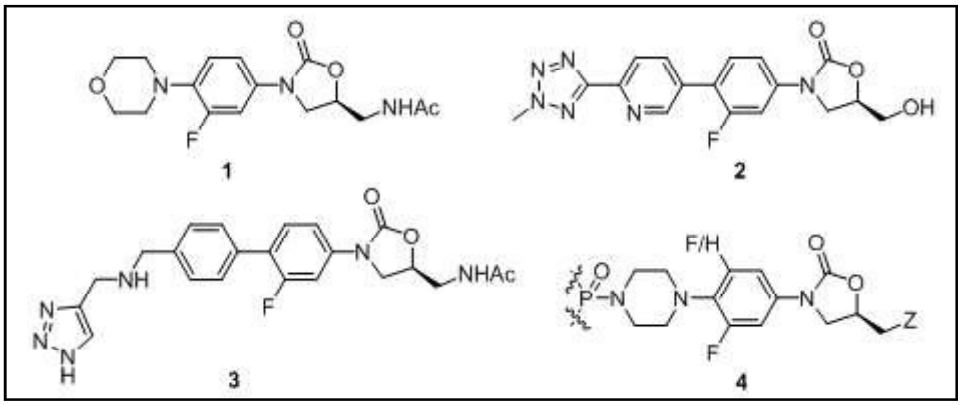
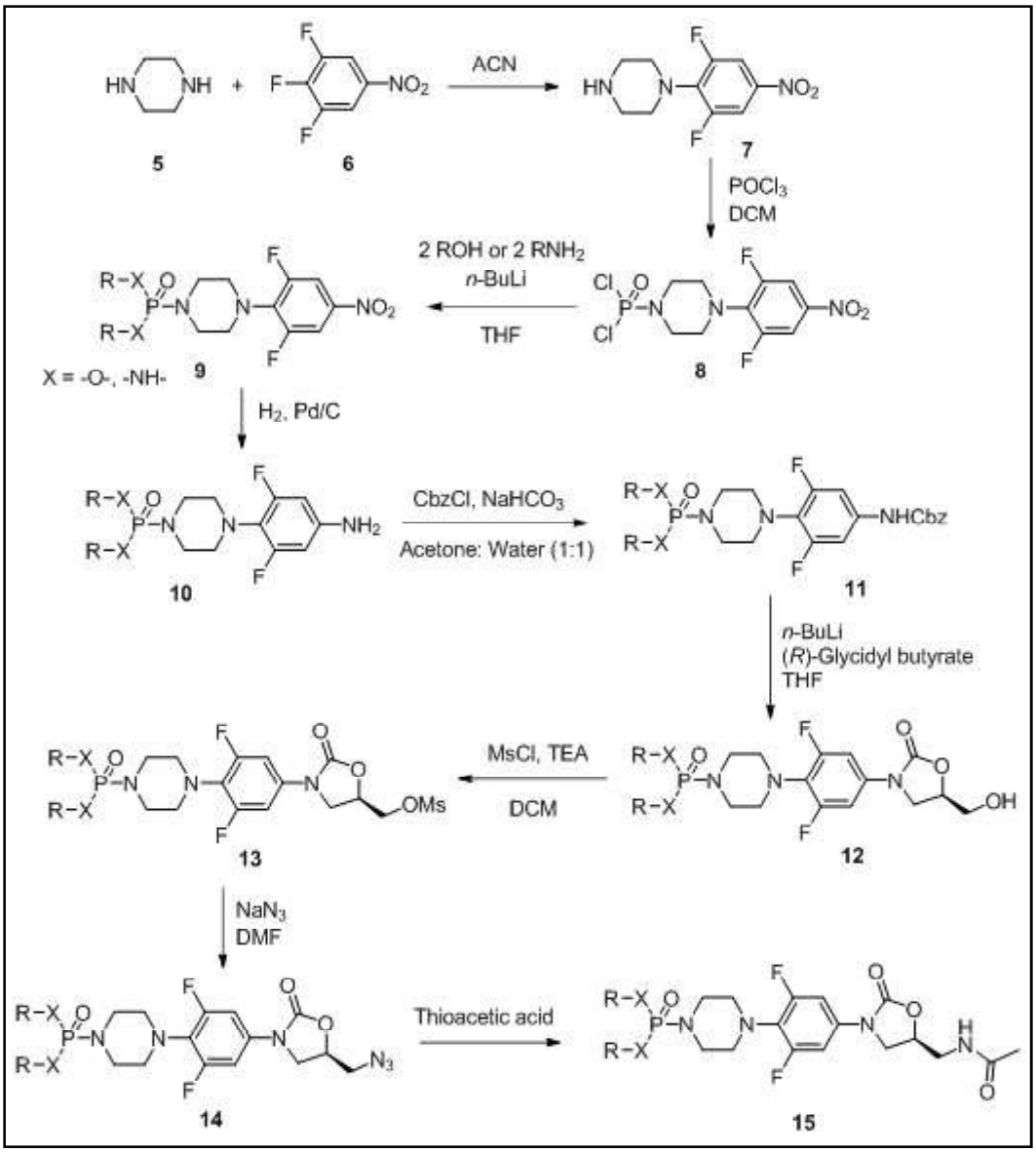


Figure 1: Oxazolidinone based antibacterials.

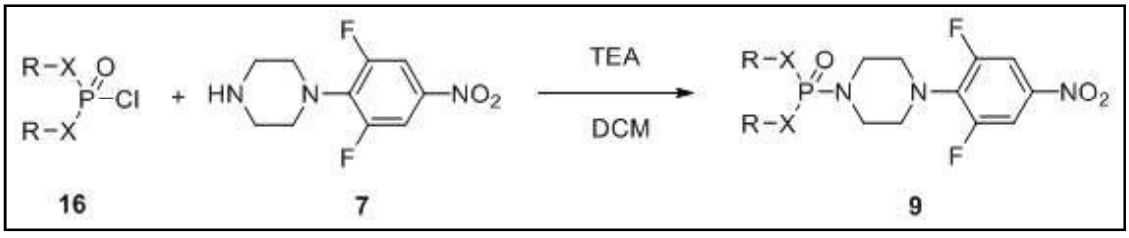


Scheme 1: Synthesis of acetamide derivatives of phosphorous substituted oxazolidinones.

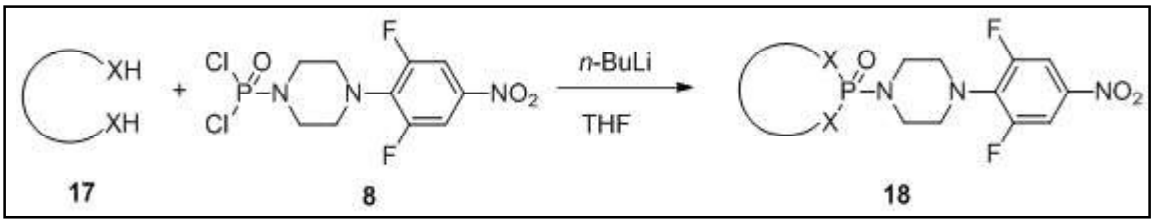
However, it took 14 years for the second molecule from oxazolidinone series, Tedizolid **2** (effective against skin infection), to come to the market and Radezolid **3** (active against LNZ resistant strains), the third molecule, is in late stage of clinical trial. Linezolid is widely employed for treating infections caused by Gram-positive bacteria. It is also effective in the treatment of drug-resistant pulmonary infections and multidrug resistant TB (MDR-TB). Many other oxazolidinones are at different stages of development (sutezolid, eperezolid, delpazolid, TBI-223) and some of them are being developed against MDR-TB. It may be mentioned here that, according to a 2014 data, newly diagnosed TB patients (about 3.3%) and previously treated TB patients (about 20%) are reported to have MDR-TB.[19]

Making structural modifications in the existing antibacterial drugs seems to be the most common approach to develop new antibacterial agents to provide novel analogues with improved biological profile.[17] We found that oxazolidinone compounds **4** (Figure 1) with the special phosphorous substitution have not been explored till now for their antibacterial acitivity.[20] We envisaged, these phosphorous containing substituents might improve activity compared to linezolid and in turn might overcome resistance associated with it. To check this, molecular docking studies were carried out using the crystal structure of 50S ribosome unit of E. coli (PDB ID: 3CPW).[21] It was observed that both linezolid and novel compounds **4** bind in the same pocket of ribosome. Interestingly, phosphorous substitution played an important role in addition to the oxazolidinone ring in binding to 50S RNA. Keeping this in mind, various oxazolidinone derivatives with phosphorous substitutions were synthesized following Scheme 1.

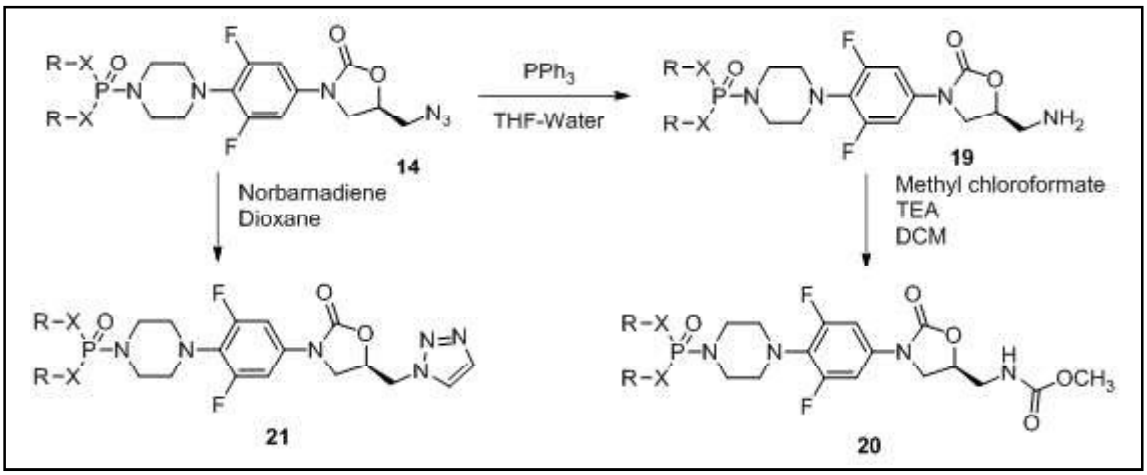
Reaction of piperazine **5** with 3,4,5-trifluoronitrobenzene **6** in acetonitrile provided nitro compound **7** as orange solid in 92% yield. Nitro compound **7** was reacted with phosphoryl chloride



Scheme 2: Alternate synthetic route for the generation of the **9**.



Scheme 3: Synthesis of cyclic phosphorous derivatives.



Scheme 4: Synthesis of carbamate and triazole derivatives.

under nitrogen atmosphere in DCM to provide phosphonic dichloride 8 as yellow solid in very good yield (70%). This novel dichloro compound is stable and can be stored under nitrogen atmosphere. Phosphonic dichloride **8** was then treated with **2** equiv. of appropriate alcohol or amine to obtain compounds **9** as yellow solids (70-80%). Nitro derivatives **9** were then reduced by hydrogenation over palladium catalyst on activated charcoal to give compounds **10** as white solid in 90-95% yield. Amines **10** upon subsequent reaction with CbzCl, in the presence of sodium bicarbonate afforded compounds **11** as white solids (90-95% yield). Oxazolidinone ring was then constructed by deprotonating **11** using n-butyllithium in THF followed by the addition of (R)-glycidyl butyrate to produce the oxazolidinones **12** (off-white solid) in 60-70% yield. Compounds **12** were reacted with methanesulfonyl chloride in DCM to afford mesylates **13** as off-white solid (90-95%). Reaction of mesylates **13** with sodium azide in DMF gave azides **14** as off-white solid in 80-85% yield. The desired acetamides **15** were prepared by reaction of azides **14** with thioacetic acid. The final compounds were mostly white solids and were obtained in 75-85% yield.

Some of the nitro derivatives **9** were prepared following Scheme 2. Commercially available compounds **16** were reacted with compound **7** using triethylamine as base in DCM to give nitro derivatives **9** as yellow solid in 85-90% yield. These nitro derivatives **9** were then converted to desired oxazolidinones **15** following procedure outlined in Scheme 1.

The cyclic phosphorous derivatives were prepared following Scheme 3. Diols or diamines **17** were reacted with dichloro derivative **8** to obtain nitro derivatives **18** as yellow solid in 70-80% yield. These nitro derivatives **18** were then converted to desired acetamide oxazolidinone antibacterial compounds following procedure outlined in Scheme 1.

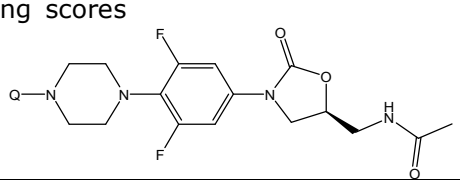
Carbamate derivatives **20** and triazole derivatives **21** were prepared following Scheme 4. Azides **14** were reduced to amines 19 using triphenyl phosphine in THF-water in 75-80% yield. Carbamate derivatives **20** were prepared by reacting amine **19** with methyl chloroformate in the presence of triethylamine as base in DCM at 0 °C. Carbamates were obtained as white solid in 90-95% yield. Triazole derivatives 21 were prepared by reacting azide derivatives **14** with norbornadiene in dioxane at 80°C as white solid (70-75%).

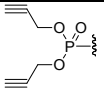
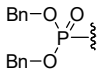
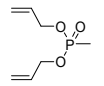
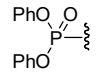
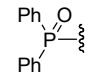
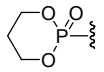
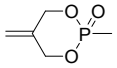
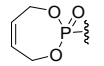
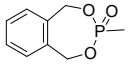
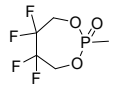
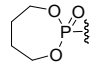
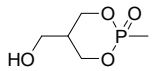
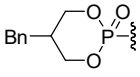
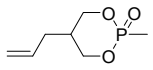
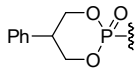
Triazole derivatives **21** were alternatively prepared by reacting mesylate **13** with potassium salt of 1,2,3-triazole in 40-45% yield.

Thus phosphorous containing oxazolidinones with various substituents either attached directly or via oxygen or nitrogen, were synthesized and screened for their in vitro antibacterial activities against a panel of resistant and susceptible Gram-positive bacteria. The results of the acetamide series are summarized in Table 1.

Most of the targeted compounds displayed very good in vitro antibacterial activities. As shown in Table 1, only a few open chain compounds **22-26** showed activities comparable to linezolid whereas almost all cyclic compounds **27-43** showed activities either comparable to or better than linezolid. All the compounds linked either through oxygen or directly through carbon showed potency equal to or better than linezolid, whereas nitrogen linked compounds

Table 1: In vitro (MIC, μg/ml) activity of novel acetamide oxazolidinones and their docking scores



Compound No.	Q	*S. aureus* ATCC 29213	*S. aureus* ATCC 33591	*E. faecalis* ATCC 29212	*E. faecium* ATTC 700221	*Docking score*
22		2	1	2	1	-7.47
23		4	2	2	1	-7.67
24		4	2	2	2	-6.85
25		2	2	2	2	-6.84
26		2	2	2	2	-5.99
27		2	2	2	2	-7.83
28		2	1	2	2	-7.61
29		2	1	1	2	-7.74
30		4	2	2	4	-7.64
31		2	1	1	2	-7.82
32		2	2	2	4	-7.34
33		4	4	2	4	-7.31
34		4	2	2	2	-6.73
35		4	1	2	2	-7.90
36		4	4	2	2	-6.92

44-46 showed weak/no antibacterial activities. Based on the activities of Table 1, a few compounds were chosen for further modification (replacement of acetamide group with methyl carbamate or 1,2,3-triazole) and their results are summarized in Table 2.

From Table 2, we can see that carbamate and triazole derivatives were either similar or more potent than their corresponding acetamides. Almost all the synthesized compounds showed antibacterial activities either comparable to linezolid or 2-4 times more potent than linezolid.

In the present series under investigation, difluoro derivatives were more effective than their corresponding monofluoro derivatives. This is evident from Table 3 (compare compounds **41** with **69, 51** and **70, 60** and **71**)

As discussed earlier, LRSA strains have started to develop resistance in clinical conditions and the next generation oxazolidinones are expected to possess activity against LRSA strains. A few oxazolidinone compounds from the present series were screened for in vitro activity against in house developed LRSA strain of ATCC 25923 and results are summarized in Table 4.

As evident from Table 4 most of these oxazolidinones showed improved activity against LRSA strain of ATCC 25923. Moreover, compounds **40, 41, 47, 58, 60, 66** were found to be 8 fold more potent than linezolid against LRSA strain of ATCC 25923.

Our next aim was to assess pharmacokinetic (pk) performances of these novel compounds. A Few compounds were selected for this purpose whose PK efficacies were checked in Swiss Albino mice by single dose (30 mg/kg) using oral route of administration and results are summarized in Table 5. From Table 5, it is evident that almost all selected compounds showed excellent

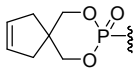
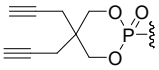
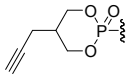
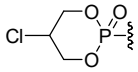
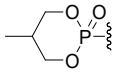
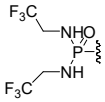
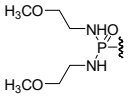
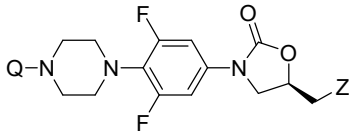
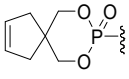
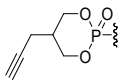
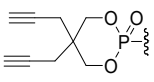
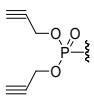
39		4	2	2	2	-7.61
40		2	1	1	2	-6.98
41		2	2	2	2	-7.58
42		4	2	2	2	7.59
43		4	2	2	2	-7.77
44		64	64	16	16	-7.91
45		64	128	32	64	-6.92
46		18	8	8	16	-7.58
1	Linezolid	2	1	2	2	**-8.32**

Table 2: In vitro (MIC, µg/ml) activity of novel oxazolidinones and their docking scores



Compound No.	Q	Z	*S. aureus* ATCC 29213	*S. aureus* ATCC 33591	*E. faecalis* ATCC 29212	*E. faecium* ATTC 700221	*Docking Score*
47		-NHCOOCH$_3$	4	2	2	2	**-8.18**
48		-NHCOOCH$_3$	2	1	2	1	-7.93
49		-NHCOOCH$_3$	2	1	1	1	-7.45
50		-NHCOOCH$_3$	4	4	4	4	-7.14
51		-NHCOOCH$_3$	1	1	1	1	-6.59
52		-NHCOOCH$_3$	1	1	1	1	-7.70
53		-NHCOOCH$_3$	2	4	4	4	-7.46

PK profile, better than linezolid in most cases. The carbamate series possessed best PK profile followed by acetamide series. Triazole compounds had PK similar to linezolid or bit inferior (Compare **27, 47** and **58**). Based on the in vitro activities and PK data, compounds having activity similar or superior to linezolid were subjected to in vivo studies in systemic infection model induced by S. aureus ATCC 29213. ED50 values were calculated after oral administration of compounds in Swiss Albino mice. Most of the compounds showed ED_{50} of ~10, the most active compound **47** showed ED50 value of 9.

To understand the observed activity of compound **47** (especially 8-fold better activity in LRSA strain compared to linezolid) and its important interactions in the active site of ribosome, molecular docking studies of compound **47** was performed in relation to linezolid using Glide software.[22] Figure 2a shows the superimposition of linezolid and compound **47** in the corresponding docking pose. Figure 2b shows the interaction between compound **47** and the ribosome.

The most important interactions of compound **47** with 50S ribosome unit are- i) Hydrogen bonding interactions of NH group (carbamate) of compound **47** with G2540 (estimated bond length 2.4 Å); ii) Hydrophobic interactions between difluoro phenyl group of compound **47** with U2541; iii) the oxazolidinone ring of compound **47** is stabilized through hydrophobic interaction with U2539 and iv) The cyclic phosphoric ester of compound **47** is stabilized through hydrophobic

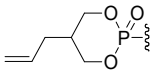
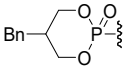
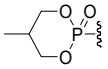
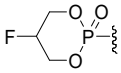
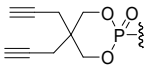
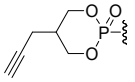
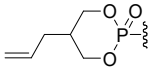
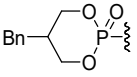
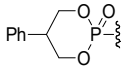
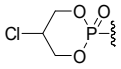
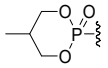
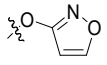
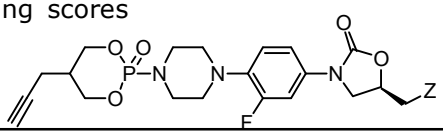
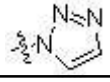
54		-NHCOOCH$_3$	4	2	2	2	-7.61
55		-NHCOOCH$_3$	4	2	2	2	-7.58
56		-NHCOOCH$_3$	4	2	2	1	7.70
57		-NHCOOCH$_3$	2	2	2	1	-7.75
58			2	1	1	1	-7.38
59			2	2	2	2	-7.80
60			2	1	1	2	-6.53
61			2	1	2	1	-7.57
62			4	2	4	2	-5.14
63			2	1	2	0.5	-7.55
64			2	1	2	1	-7.50
65			2	0.5	2	1	-4.66
66			1	1	1	1	-7.52
67			4	2	4	2	-7.22
68			2	2	2	1	-6.26
1	Linezolid		2	1	2	2	-8.32

Table 3: In vitro (MIC, µg/ml) activity of novel monofluoro oxazolidinone and their docking scores



Compound No.	Z	S.aureus ATCC 29213	S. aureus ATCC 33591	E. faecalis ATCC 29212	E. faecium ATTC 700221	Docking Score
69	-NHCOCH$_3$	8	4	2	2	-7.58
70	-NHCOOCH$_3$	8	4	4	4	-7.84
71		4	4	4	2	-7.46

interaction with G2618. Thus, cyclic phosphoric ester group played a pivotal role in addition to oxazolidinone ring in the pharmacodynamics of the ligand in the active site, as evident from additional interaction seen in the docking study.[22] Also, compound **47** showed best docking score compared to all the synthesized compounds (Tables 1-3, last column) and interestingly, was the most active compound among all the synthesized compounds.

Figure 2: a) overlapping of linezolid (yellow) and Compound **47** (red), b) 3D interaction diagram for Compound **47** in the binding pocket of ribosome. Black dots represent the H-bonding and the distances are in Å (Preference of color: online only)

Conclusion:

In conclusion, we have been able to identify a novel class of antibacterial agents, phenyl oxazolidinones, with special phosphorous substitution. To the best of our knowledge, this is the first report of phosphorous substituted phenyloxazolidinones showing excellent antibacterial activities. The diversity of functionality, tolerated on phosphorous atom, was remarkable. Potent activities were exhibited by most of the target molecules against Gram-positive sensitive as well as resistant strains. Compound **47**, having an 8-fold better activity in LRSA strain compared to linezolid, displayed otstanding PK profile and has been shortlisted for further safety evaluation. Docking studies also reveal additional interactions between these novel

Table 4:In vitro (MIC, µg/ml) activity of selected compounds against LRSA strain of ATCC 25923

Compound No.	LRSA strain of ATCC 25923	Compound No.	LRSA strain of ATCC 25923
27	16	**52**	16
29	16	**54**	16
35	16	**56**	16
39	16	**58**	8
40	8	**60**	8
41	8	**66**	8
47	8	**67**	16
49	16	**1** (Linezolid)	64

Table 5: Pharmacokinetic (pk) parameters of selected compounds.

Compound No.	$AUC_{(0-t)}$ (mg.h/ml)	$T_{1/2}$ (h)	C_{max} (mg/ml)	T_{max} (h)
22	68.58	1	15.01	0.25
27	33.08	2.49	7.44	1
29	114.26	0.91	27.86	0.25
39	18.97	0.68	9.032	0.25
41	33.31	1.5	10.98	0.25
47	67.67	4.2	13.65	1
51	48.36	3	17.94	0.25
54	56.20	3.1	19.6	0.5
58	14.13	1.45	6.65	0.5

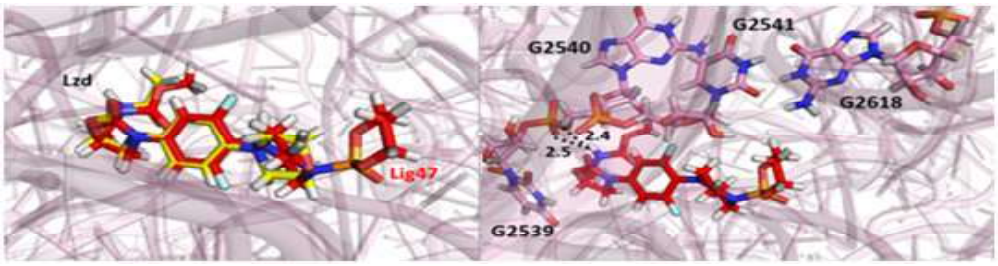


Figure 2: a) overlapping of linezolid (yellow) and Compound **47** (red), b) 3D interaction diagram for Compound **47** in the binding pocket of ribosome. Black dots represent the

phosphorous compounds and the ribosome, compared to linezolid.

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Tribute - We are deeply saddened by the untimely demise of Dr. Sandeep Kanwar (an author of this article), a sincere and budding medicinal chemist, last year.

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