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Effects of positional and geometrical isomerism on the biological activity of some novel oxazolidinones☆,☆☆

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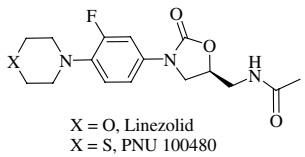
Abstract—Some novel oxazolidinone derivatives with benzotriazole as pendant have been synthesized and tested for antibacterial activity. Linearly attached benzotriazole derivative showed more potency compared to angular one in vitro. Out of *E*/*Z*-isomers of angularly attached derivatives *E*-isomer was found to be more potent than *Z*-isomer. Either less active or inactive molecules were obtained, when benzotriazole was replaced with benzimidazole, benzthiazole, or benzoxazole. Finally, thioacetamide analogue of linear compound gave a lead having activity similar to linezolid in vitro.

1. Introduction

Tuberculosis is one of the leading causes of death due to infection worldwide. Today, nearly one third of world population is infected with *Mycobacterium tuberculosis*; and according to WHO report this number is increasing every year.[1] Both the developed and developing countries are facing a challenge to combat this disease. The complexity is multifold in the case of immunocompromised patients acquiring *Mycobacterium avium* complex (MAC) infections. The emergence of multidrug resistant (MDR) *M. tuberculosis* poses a serious threat to the current therapies used for the treatment of tuberculosis. Therefore, there is an urgent need to develop novel drugs for tuberculosis.

Oxazolidinones, a completely synthetic antibacterial class, elicited lots of interest amongst medicinal chemists because of its activity against resistant Gram-positive organisms such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococci* (VRE), and glycopeptide intermediate resistant *S. aureus* (GISA). Oxazolidinones bind selectively to 50S ribosomal subunit thereby inhibiting bacterial protein biosynthesis at an early stage.[2] Linezolid is the first drug from this class of compounds launched in 2000 by Pharmacia. Subsequently it was tested against 249 clinical isolates of rapidly growing mycobacteria and MIC of 4 μg/mL was reported against most of the strains studied.[3] However, thiomorpholine analogue of linezolid, PNU 100480 (Fig. 1) was found to be more potent having MIC of 0.125 μg/mL against mycobacterial strains.[4]

We have a long-standing interest to develop drugs for tuberculosis and oxazolidinone class fits well into our objectives for its unique mechanism of action. Moreover oxazolidinones are completely synthetic class of molecules having less possibility of developing resistance.[5]



X = O, Linezolid
X = S, PNU 100480

Figure 1.

Keywords: Oxazolidinones; *E*–*Z*-isomers; Positional isomers; Antibacterial; Benzotriazole.
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X,Y,Z = N, C; M = 2 H, CR_2, CHR, PhCH, p-$NO_2C_6H_4CH$, p-$NR_2C_6H_4CH$ etc.

Figure 2.

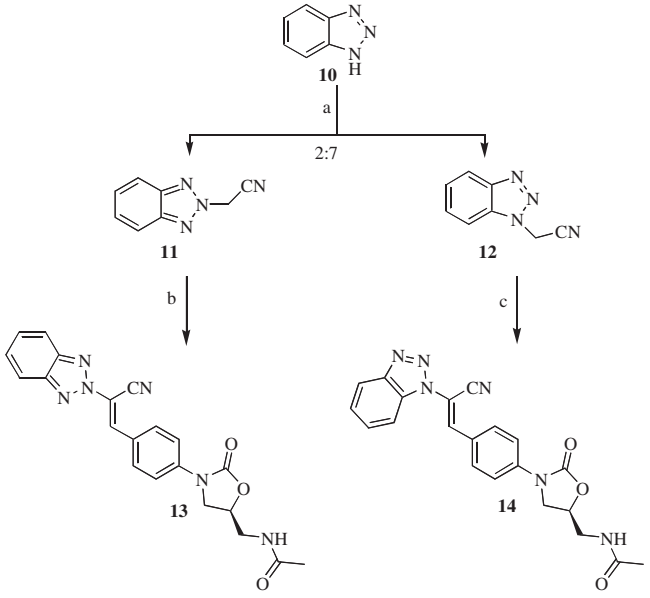
Literature search revealed the following common structures having antituberculosis activity (Fig. 2).[6,7]

The combination of two antibacterial agents with different mechanisms of action into a single entity to achieve drugs with dual action has received considerable attention in the literature.[8] We deliberated on the above chemical structures and decided to hybridize them with oxazolidinones to explore the antituberculosis activity of the resulting molecules (Fig. 3).

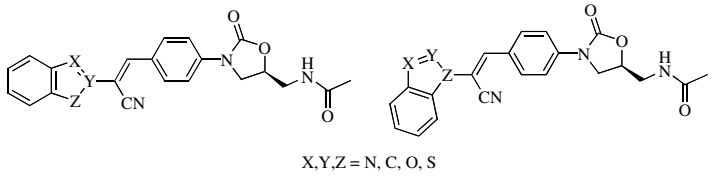
2. Chemistry

The key step involved in the synthesis of this series of compounds is the Knoevenagel condensation of aldehyde **1** with acetonitrile derivative **2** to give the hybrid molecule **3** (Scheme 1).

Synthesis of aldehyde **1** has been depicted in Scheme 2. Reduction of both nitro and aldehyde groups of 4-nitrobenzaldehyde was achieved with $NiCl_2$ and $NaBH_4$ to obtain the amino alcohol **5**. Protection of amine as benzylcarbamate and alcohol as TBDMS ether gave

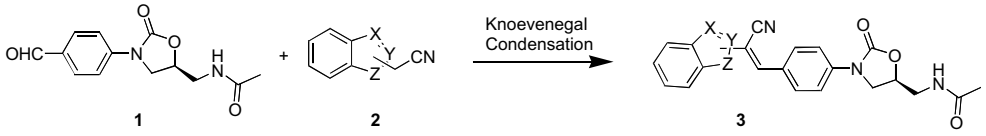


Scheme 3. Reagents and conditions: (a) $ClCH_2CN$, TEA, DMF, 110 °C, 50%; (b) ald. **1**, benzoic acid, piperidine, toluene, reflux, 40%; (c) same as (b), 50%.

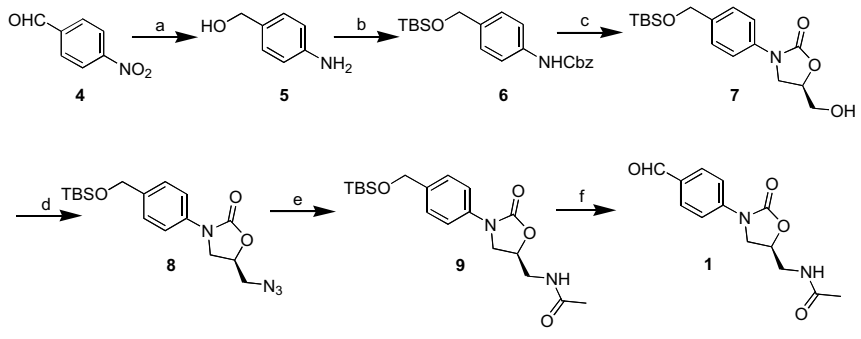


X,Y,Z = N, C, O, S

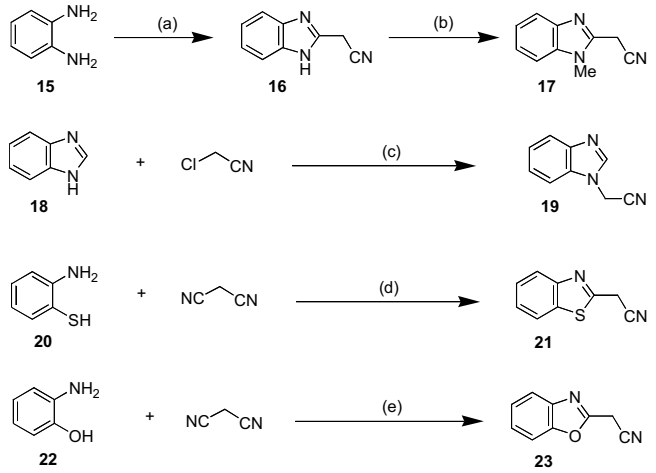
Figure 3.



Scheme 1.



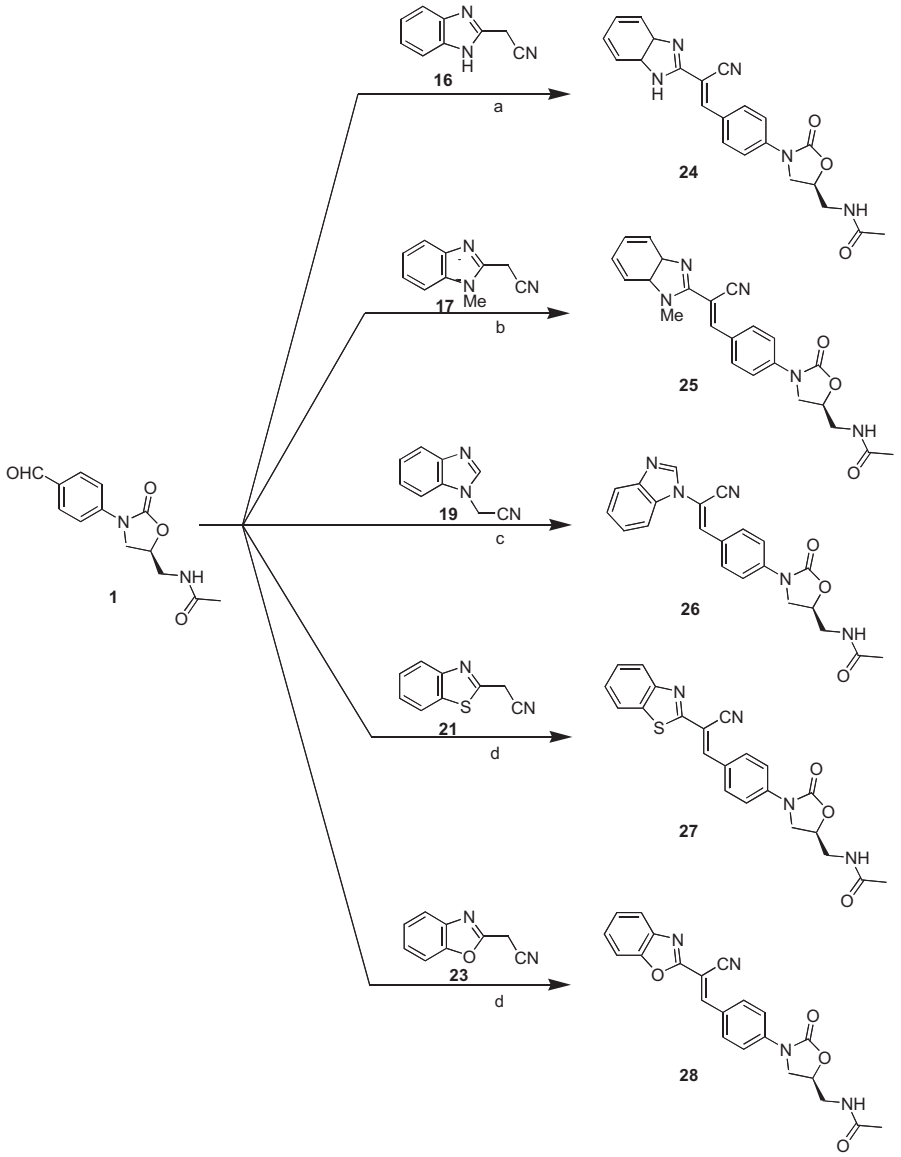
Scheme 2. Reagents and conditions: (a) $NaBH_4$, $NiCl_2$, 71%; (b) 1. Cbz–Cl, $NaHCO_3$, 2. TBDMS–Cl, Im, 43%; (c) *n*-BuLi, (*R*)-glycidyl butyrate, −78 °C to rt, 55%; (d) 1. MsCl, TEA, 2. NaN_3, 76%; (e) MeCOSH, 66%; (f) 1. TBAF, 2. PDC, 63%.



Scheme 4. Reagents and conditions: (a) $CNCH_2COOH$, 4N HCl, 50%; (b) K_2CO_3, DMF, MeI, 75%; (c) TEA, DMF, 50%; (d) acetic acid/ethanol, 20%; (e) same as (d), 35%.

compound **6**. This compound was converted to **9** following well known methods in the literature.[9] Deprotection of TBDMS ether and subsequent oxidation of the resulting alcohol with PDC produced the required aldehyde **1**.

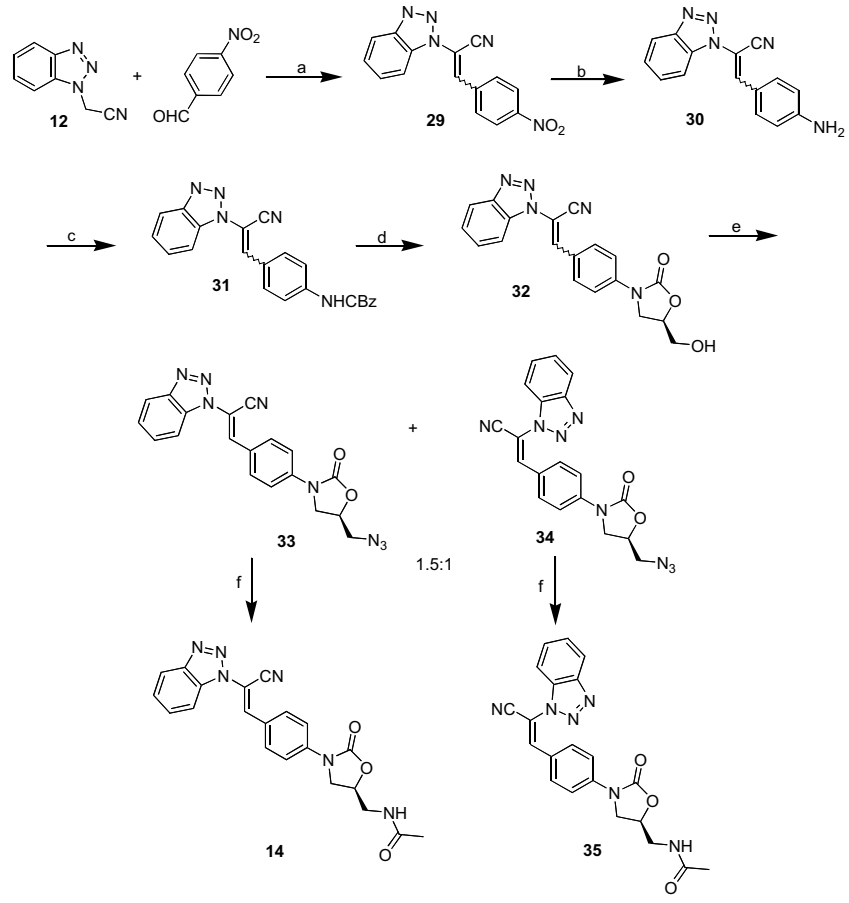
Initially we decided to synthesize benzotriazole derivatives **3** (Scheme 1, X = Y = Z = N) because both the regioisomers of benzotriazole **11** and **12** are readily available and a systematic study of *E*- and *Z*-isomers involving **11** and **12** with substituted benzaldehydes have been carried out by Sanna et al.[7] Acetonitrile derivatives **11** and **12** were obtained from benzotriazole and chloroacetonitrile in 2:7 ratio.[7] These two compounds were separated by column chromatography and spectral data were compared with the reported values. Compounds **11** and **12** were then condensed with aldehyde **1** under Knoevenagel conditions to give **13** and **14**, respectively, and only *E*-isomers were isolated in both the examples (Scheme 3).



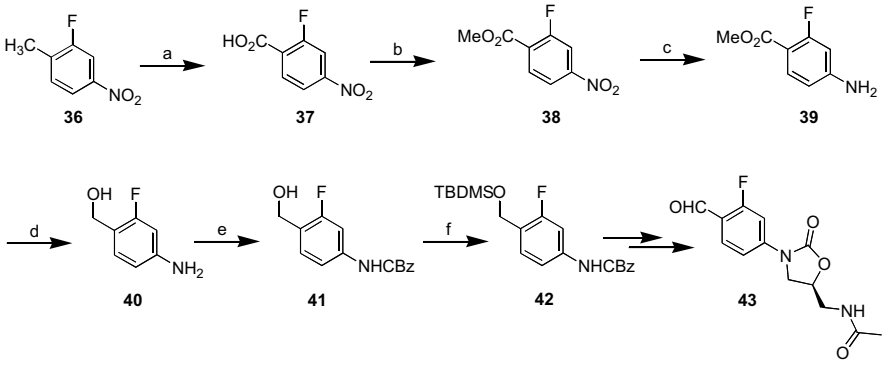
Scheme 5. Reagents and conditions: (a) KF, dry ethanol, 52%; (b) same as (a), 41%; (c) TEA, toluene, reflux, 50%; (d) CsF, dry ethanol, 15%.

Various acetonitrile derivatives were then prepared for condensation with aldehyde **1** (Scheme 4). 1,2-Phenylenediamine was converted to *C*-linked imidazol derivative **16** by heating with cyanoacetic acid in 4 N HCl, which was further methylated with methyl iodide to give compound **17**. *N*-Linked imidazol derivative **19** was prepared from imidazol and chloroacetonitrile in DMF with triethyl amine as base. Benzthiazole derivative **21** and benzoxazole derivative **23** were synthesized from *o*-aminothiophenol **20** and *o*-aminophenol **22**, respectively, with malononitrile in ethanol and acetic acid.

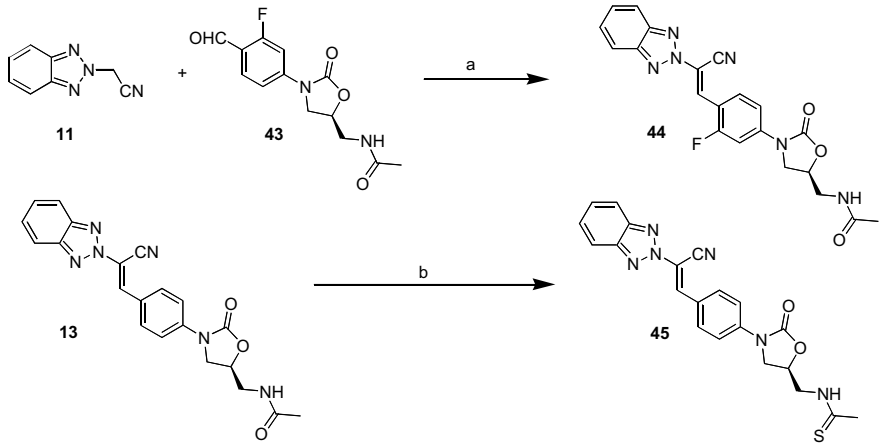
Condensation of acetonitrile derivatives with aldehyde **1** under Knoevenagel condition was tried with different base/solvent combinations. However, Knoevenagel condensation was base sensitive and a single base/solvent combination could not be utilized for all the conversions.[10] Compound **16** was condensed with aldehyde **1**



Scheme 6. Reagents and conditions: (a) TEA, toluene, 50%; (b) $NiCl_2/NaBH_4$, 84%; (c) CbzCl, $NaHCO_3$, 57%; (d) *n*-BuLi, (*R*)-glycidyl butyrate, −78 °C to rt, 50%; (e) 1. MsCl, TEA, 2. NaN_3, DMF, 51%; (f) CH_3COSH, 65%.



Scheme 7. Reagents and conditions: (a) $Na_2Cr_2O_7$, H_2SO_4, 90 °C, 70%; (b) MeOH, H_2SO_4, 93%; (c) PtO_2/H_2, 95%; (d) LAH, 60%; (e) CbzCl, $NaHCO_3$, 93%; (f) TBDMSCl, TEA, DMAP, 63%.



Scheme 8. Reagents and conditions: (a) KF/dry ethanol, 15%; (b) Lawesson's reagent, dioxane, 41%.

to produce **24** in dry ethanol and KF. Similarly compound **17** gave **25** under identical conditions whereas compound **26** was obtained from **19** and aldehyde **1** with triethylamine as base. Compounds **21** and **23** could not be condensed under both the conditions. However, compounds **27** and **28** could be obtained in modest yields on treatment with CsF in dry ethanol (Scheme 5).

Z-Isomer of **14** was prepared following the sequence described in Scheme 6. Knoevenagel condensation of **12** with 4-nitrobenzaldehyde gave a mixture of *E*- and *Z*-isomers **29**. The mixture of isomers was reduced to the corresponding mixture of amines **30** with $NiCl_2{\cdot}6H_2O$ and $NaBH_4$. Isomeric amines **30** were converted to corresponding azidomethyl oxazolidinones **33** and **34** in 3:2 ratio following the known sequence of reactions (Scheme 2); and at this stage isomers could be separated by column chromatography. Finally, both *E*- and *Z*-isomers **14** and **35** were obtained from azides **33** and **34**, respectively, on treatment with thiolacetic acid.

Synthesis of fluoro derivative of **13** is described in Scheme 7. 2-Fluoro-4-nitro toluene **36** was oxidized to 2-fluoro-4-nitro benzoic acid **37**, which was then esterified with methanol to give **38**. Reduction of both ester and nitro groups of **38** to amino alcohol **40** in one pot could not be accomplished. Therefore reduction of nitro group to amine **39** was carried out with PtO_2/H_2 and then to alcohol **40** by lithium aluminum hydride. Amino group of compound **40** was protected as benzylcarbamate (**41**) and hydroxy group as TBDMS ether to give compound **42**. Aldehyde **43** was then obtained following the same procedure for aldehyde **1**.

Condensation of acetonitrile derivative **11** with aldehyde **43** in presence of KF in dry ethanol yielded **44**, the fluoro analogue of **13**. In a separate reaction, **13** was converted to thioacetamide derivative **45** using Lawesson's reagent (Scheme 8).

It is our observation that Knoevenagel condensation of aromatic aldehydes with the acetonitrile derivatives discussed here produced in most of the cases the *E*-isomer as the only isolable product. *E*-Isomers have been characterized by higher δ values for vinylic protons[7] in ^{1}H NMR and also by $^3J_{C-H}$ (13–14 Hz) coupling for vinylic H and nitrile C by using the 'gate decoupling' technique.[11] The ^{1}H NMR values for vinylic protons of all the compounds have been compiled in Table 1. The vinylic proton peaks in ^{1}H NMR appeared at δ 8.21–8.70 for all the *E*-isomers in accordance with the reported values except compound **14**. In this case the vinylic proton appeared at δ 6.08. The upfield shift of vinylic proton for compound **14** was abnormal compared to other *E*-isomers. However, the vinylic proton for *Z*-isomer was found to appear at δ 6.22 as expected. The reason for the abnormal behavior of compound **14** is not clear to us at this moment. In order to prove that compound **14** is really *E*-isomer we have isolated both *E*- and *Z*-isomers from mixtures of **29** (Scheme 6) by careful column chromatography along with mixture of *E*- and *Z*-isomers and identified them on the basis of spectral data reported.[7] Both the isomers were then converted to **14** and **35** following the same sequence of reactions as depicted in Scheme 6 independently.

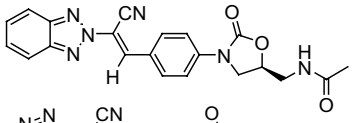
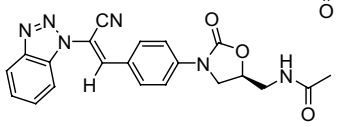
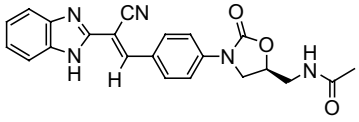
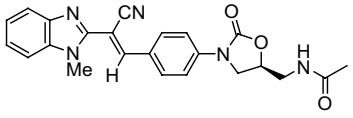
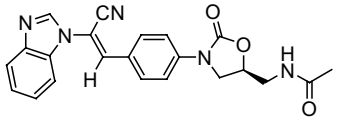
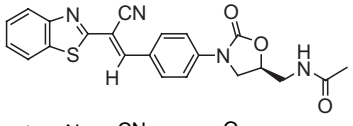
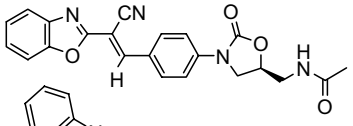
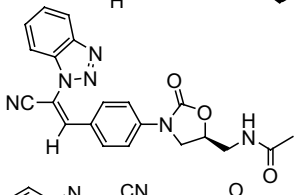
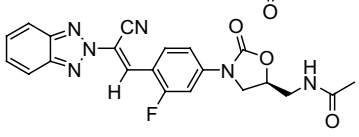
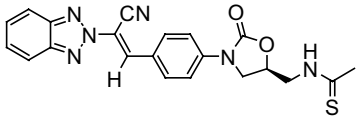
3. Biology

All the compounds listed in Table 1 were tested against *Mycobacterium smegmatis* MTCC 006 in addition to the panel of organisms consisted of six reference Gram-positive bacteria including both sensitive and resistant strains: *S. aureus* ATCC 29213 (MSSA), *S. aureus*

Table 1. ^{1}H NMR (δ) values for vinylic protons

Entry	Compd no	δ values
1	**13**	8.55
2	**14**	6.08
3	**35**	6.22
4	**24**	8.31
5	**25**	8.22
6	**26**	8.45
7	**27**	8.21
8	**28**	8.32
9	**44**	8.70
10	**45**	8.52

Table 2. In vitro potency of oxazolidinones

Entry	Compd no	Compound structure	MIC (μg/mL)						
			M.sm[a] 006	*S.a* 29213	*S.a* 49951	*S.a* 33591	*E.f* 29212	*E.f* 12201	*E.fm* 12202
1	**13**		>32	8	8	8	8	8	8
2	**14**		>32	16	16	16	32	32	32
3	**24**		>32	>32	>32	>32	>32	>32	>32
4	**25**		>32	>32	>32	>32	>32	>32	>32
5	**26**		>32	16	16	16	32	32	32
6	**27**		>32	>32	>32	>32	>32	>32	>32
7	**28**		>32	>32	>32	>32	>32	>32	>32
8	**35**		>32	>32	>32	>32	>32	>32	>32
9	**44**		>32	8	16	16	32	32	32
10	**45**		**>32**	**2**	**2**	**2**	**4**	**4**	**4**
11		Linezolid	2	2	2	2	2	2	2
12		Streptomycin	0.5	4	2	>32	>32	>32	32

[a] *M.sm* 006: *Mycobacterium smegmatis* MTCC 006; *S.a* 29213: *S. aureus* ATCC 29213 (MSSA); *S.a* 49951: *S. aureus* ATCC 49951 (Smith strain); *S.a* 33591: *S. aureus* ATCC 33591 (MRSA); *E.f* 29212: *E. faecalis* ATCC 29212; *E.f* 12201: *E. faecalis* NCTC 12201 (VREf); *E.fm* 12202: *E. faecium* NCTC 12202 (VREfm).

ATCC 49951 (Smith), *S. aureus* ATCC 33591 (MRSA), *E. faecalis* ATCC 29212, *E. faecalis* NCTC 12201 (VREf) *E. faecium* NCTC 12202 (VREfm). MIC was determined by brothmicro dilution as per the guidelines prescribed by NCCLS.[12] Doubling dilution of the test compound, in the range of 32–1 μg/mL, was carried out in Mueller Hinton Medium (Difco). The organisms were inoculated to obtain a final concentration of 1–5×10^5 cfu/mL and the microtiter plates were incubated overnight, in ambient air, at 35 °C. Linezolid and streptomycin were included as controls.

4. Results

Our hypothesis to hybridize oxazolidinones with fragments having antituberculosis activity to achieve better molecules for the treatment of tuberculosis was found to be illusive. None of the compounds tested showed antibacterial activity against *M. smegmatis* (Table 2). However, some of these compounds were found to be active against the Gram-positive strains mentioned above. Benzotriazole derivative **13**, having attachment through middle nitrogen (linear attachment), exhibited

moderate activity (MIC, 8 μg/mL). On the other hand compound **14**, a positional isomer of **13** (having attachment through terminal nitrogen, i.e., angular attachment), was found to be less active than the linear one. *C*-Linked benzimidazole derivative **24** and its methyl derivative **25** did not show antibacterial activity against the strains tested whereas *N*-linked benzimidazole derivative **26** retained the activity of analogues compound **14**. Derivatives of benzthiazole **27** and benzoxazole **28** were found to be inactive against the strains tested. Compound **35**, *Z*-isomer of **14** was found to be inactive too. It is a general observation in oxazolidinone series that replacement of hydrogen of central aromatic ring with fluorine, having *meta* relationship with oxazolidinone group, is beneficial as far as MIC is concerned. However, in this series compound **44**, fluoro analogue of **13**, showed reduced antibacterial activity. On the other hand, thioacetamide analogue (**45**) of **13** was found to poses good antibacterial activity (MIC, 2–4 μg/mL) and was the most potent amongst the molecules screened.

5. Conclusion

We have synthesized various oxazolidinone derivatives having positional and geometrical substitutions with benzotriazole, benzimidazole, benzthiazole, and benzoxazole. Contrary to our expectations none of the compounds showed antimycobacterial activity. However, some of them showed antibacterial activity against Gram-positive pathogens. Compound having linear attachment with benzotriazole was found to be the most potent among the compounds tested. Deviation from the position of attachment or the geometry of the molecule has dramatic effect on the activity. Heterocycle, other than benzotriazole, was also not tolerated. Finally, a lead molecule having activity comparable to linezolid has been identified.

Acknowledgements

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