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PII: S0223-5234(16)30548-7

DOI: 10.1016/j.ejmech.2016.07.001

Reference: EJMECH 8719

To appear in: *European Journal of Medicinal Chemistry*

Received Date: 10 March 2016

Revised Date: 16 May 2016

Accepted Date: 2 July 2016

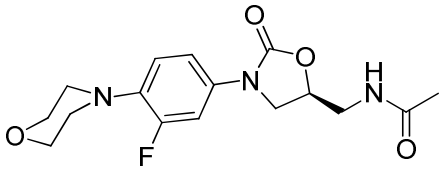
Please cite this article as: P.K. Gadekar, A. Roychowdhury, P.S. Kharkar, V. Khedkar, M. Arkile, H. Manek, D. Sarkar, R. Sharma, V. Vijayakumar, S. Sarveswari, Design, synthesis and biological evaluation of novel azaspiro analogs of linezolid as antibacterial and antitubercular agents, *European Journal of Medicinal Chemistry* (2016), doi: 10.1016/j.ejmech.2016.07.001.

For Table of Contents Use Only

Design, Synthesis and Biological Evaluation of Novel Azaspiro Analogs of Linezolid as Antibacterial and Antitubercular Agents

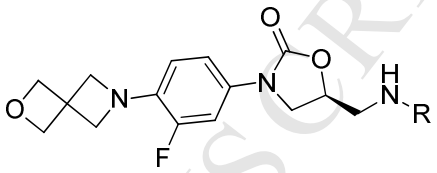
Pradip K. Gadekar[a], Abhijit Roychowdhury[a], Prashant S. Kharkar[b], Vijay Khedkar[c,d], Manisha Arkile[c], Hardik Manek[c], Dhiman Sarkar[c], Rajiv Sharma[a], V.Vijayakumar[e] S. Sarveswari[e]*

[a]Department of Medicinal Chemistry, Piramal Enterprises Ltd., 1, Nirlon Complex, Goregaon (East), Mumbai –400063, [b]Department of Pharmaceutical Chemistry, SPP School of Pharmacy and Technology Management, SVKM's NMIMS, V. L. Mehta Road, Vile Parle (West), Mumbai – 400 056; [c]Combi Chem-Bio Resource Centre, Division of Organic Chemistry, CSIR-National Chemical Laboratory, Dr. Homi Bhabha Road, Pune – 411 008. INDIA. [d]School of Health Sciences, University of KwaZulu Natal, Westville Campus, Durban 4000, South Africa [e]Center for Organic and Medicinal Chemistry, School of Advanced Sciences, VIT University, Vellore – 632014, Tamil Nadu, INDIA.



Linezolid

IC_{50} = 0.8 µg/mL (MtbH37a Active)
IC_{50} = 0.71 µg/mL (MtbH37a Dormant)



18-42

IC_{50} = 1.56 – 16.34 µg/mL (MtbH37a Active)
IC_{50} = 2.07 – 22.83 µg/mL (MtbH37a Dormant)

Design, synthesis and biological evaluation of novel azaspiro analogs of linezolid as antibacterial and antitubercular agents

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[a]*Department of Medicinal Chemistry, Piramal Enterprises Ltd., 1, Nirlon Complex, Goregaon (East), Mumbai –400063. INDIA.*

[b]*Department of Pharmaceutical Chemistry, SPP School of Pharmacy and Technology Management, SVKM's NMIMS, V. L. Mehta Road, Vile Parle (West), Mumbai – 400 056. INDIA.*

[c]*Combi Chem-Bio Resource Centre, Division of Organic Chemistry, CSIR-National Chemical Laboratory, Dr. Homi Bhabha Road, Pune – 411 008. INDIA.*

[d]*School of Health Sciences, University of KwaZulu Natal, Westville Campus, Durban 4000, South Africa*

[e]*Center for Organic and Medicinal Chemistry, School of Advanced Sciences, VIT University, Vellore – 632014, Tamil Nadu, INDIA.*

† Electronic Supplementary Information (ESI) available: Spectral data

*Authors for correspondence

Dr. S. Sarveswari, M. Sc., M. Phil., Ph. D.,

Center for Organic and Medicinal Chemistry, School of Advanced Sciences, VIT University, Vellore – 632014, Tamil Nadu, INDIA

*E-mail: sarveswari@gmail.com

Tel: +91-9042407099

ABSTRACT

The design, synthesis and antimicrobial evaluation of a novel series of azaspiro analogues of linezolid (**1**) have been described. Linezolid comprises of a morpholine ring which is known for its metabolism-related liabilities. Therefore, the key modification made in the linezolid structure was the replacement of morpholine moiety with its bioisostere, 2-oxa-6-azaspiro[3.3]heptane. Furthermore, the replacement of *N*-acetyl terminal of **1** with various aromatic or aliphatic functionalities was carried out. The title compounds were evaluated against a panel of Gram-positive and Gram-negative bacteria and *Mycobacterium tuberculosis*. Subsequent structure-activity relationship (SAR) studies identified several compounds with mixed antibacterial and antitubercular profiles. Compound **22** (IC_{50} 0.72, 0.51, 0.88, 0.49 µg/mL for *E. coli*, *P. aeruginosa*, *S. aureus*, *B. subtilis*, respectively) exhibited similar antibacterial profile as **1**. The *N*-acetyl derivative **18** was similar to **1** in antitubercular profile. Thus, the present study successfully demonstrated the use of azaspiro substructure in the medicinal chemistry of antibacterial and antitubercular agents.

KEYWORDS

Azaspiro, MRSA (methicillin-resistant *Staphylococcus aureus*), bioisostere, morpholine, antibacterial, antitubercular, VRE (vancomycin-resistant *enterococci*)

1. Introduction

The bacterial resistance to established antimicrobial drugs has become a critical global problem. Infections caused by multidrug-resistant Gram-positive cocci, such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *enterococci* (VRE) and penicillin-resistant *Streptococcus pneumoniae* (PNSSP), etc., have emerged as major public health concern [1]. The number of effective antibiotics has reduced drastically, because microorganisms are able to create new mechanisms of resistance and rapidly spread the genes encoding them via mobile genetic elements, mostly plasmids and integrons [2]. Similarly, tuberculosis (TB) also remains a major global health challenge and has been declared as public health emergency by the World Health Organization (WHO) [3]. TB affects one-third of the world population. About 9 million new cases and 1.1 million TB-related deaths were reported in 2013 [3]. Multidrug-resistant strains of *Mycobacterium tuberculosis* (Mtb), the causative agent of TB (MDRTB) and extremely drug-resistant TB (XDRTB) have emerged [4,5]. There is an extremely urgent need of new antitubercular drugs which can tackle the TB menace.

As a prerequisite, newer antitubercular drugs should be active against the drug-resistant forms of Mtb. This implies that they must act preferably on molecular targets different than those of the current drugs. Importantly, such drugs need to be more active against persistent Mtb, which may lead to further reduction in the treatment duration. The treatment of TB is complicated by the tendency of its etiological agent, predominantly Mtb, to adopt a nonreplicating persistent state [6-8].

Linezolid (**1**, Zyvox®, Figure 1), a newer generation synthetic antibiotic from oxazolidinone class, was developed by a team at Pharmacia and Upjohn Company (now part of Pfizer) [9]. It was approved in 2000 for the treatment of serious infections caused by Gram-positive bacteria resistant to several other antibiotics such as MRSA, VRE (responsible for soft tissue infections and PNSSN) [10]. Currently, **1** and related compounds are under clinical investigation for the treatment of TB (Figure 1) [11]. Recently, cytoxazone-linezolid hybrids were shown to induce apoptosis and senescence in DU145 prostate cancer cell line [12a].

The mechanism of action of **1** involves interaction with 50S A-site pocket at the peptidyl transferase center (PTC) of the bacterial ribosome, which overlaps with the aminoacyl moiety of an A-site bound tRNA, thereby inhibiting overall protein synthesis [12b,13]. However, in some

cases, linezolid-resistant *S. aureus* and *Enterococci* were found among the hospital isolates [14,15]. Toxic effects of linezolid on prolonged use include reversible myelosuppression leading to anaemia, leucopaenia and thrombocytopaenia. In addition to this, **1** has been shown to inhibit monoamine oxidase (MAO) enzymes, potentially leading to drug-drug interactions with adrenergic and serotonergic agents [2]. Overall, newer and potent antibiotics are expected to possess: i) extended spectrum of antibacterial activity covering fastidious Gram-negative organisms; ii) activity against linezolid-resistant strains; iii) improved safety profile leading to circumvention or at least minimization of myelosuppression and iv) activity against drug-resistant forms of Mtb.

In the present investigation, bioisosteric modifications of **1** were tried in order to develop potential new treatment against drug-resistant forms of bacteria, including Mtb, with reduced toxicity profiles. Here, replacement of the morpholine ring in **1** with 2-oxa-6-azaspiro[3.3]heptane was attempted. Morpholine is one of the common privileged structures found in several drugs such as linezolid, timolol, gefitinib, moracizine, nimorazole, emorfazone, etc. It is often used to raise aqueous solubility of drugs.

However, morpholine ring is regularly a target of the oxidative metabolism by non-cytochrome P450 (CYP) enzyme systems. This is evident from the oxidative metabolites reported for at least eight marketed drugs containing morpholine [16-18]. The 2-oxa-6-azaspiro[3.3]heptanes, a bioisostere of morpholine (Figure 2) was selected for combating the oxidative metabolism issues associated with morpholine. The azaspiro analogue of morpholine, 2-oxa-6-azaspiro[3.3]heptanes, is better due to its improved chemical stability, lower lipophilicity, higher solubility and metabolic robustness over morpholine [19]. The present study documents a systematic structure-activity relationship (SAR) investigation of the azaspiro analogue of **1** (compound **18**, Table 1, 2) leading to potent antibacterial and antitubercular agents.

<< Figure 1 >>

<< Figure 2 >>

2 Results and discussion

2.1 Chemistry

The azaspiro intermediates **9** and **10** were synthesized by reported method [20] in good yield. Condensation of **10** with 3,4-difluoronitrobenzene (**11**) under refluxing conditions in presence of DIPEA in ACN led to compound **12** which was subjected to catalytic hydrogenation to yield substituted aniline **13**. It was further reacted with commercially available **14** to afford secondary alcohol **15**. Use of DIPEA in DMF at 120 °C resulted in moderate yield compared to other bases and polar solvents (data not shown). Further, **15** was cyclized to oxazolidinone **16** using CDI, followed by deprotection of the pthalimide-protected 5-methylamino group using hydrazine hydrate to get alkylamine compound **17**.

The systematic exploration of the SAR at the 5-methylamino group of **17** (amide, sulfonamide, urea and thiourea derivatives) yielded a library of title compounds (see Supporting Information section) on reaction with substituted acid chlorides, sulphonylchlorides, isocyanates and thioisocyanates, respectively, at 0-5 °C for 2 hrs. The crude products were purified using flash chromatography (2-5% MeOH in DCM) to afford title compounds **18-42** (Scheme 1). Compound **45** was synthesized by condensing commercially available intermediates **43** and **44** at 100 °C in presence of 2M Na_2CO_3, $PdCl_2$ (dppf)-CH_2Cl_2 adduct in 1,4-dioxane (Scheme 2).

Further, we have synthesized novel azaspiro compound **52** which was central spiro ring analogue of **1** (Scheme 3). Compound **47** was synthesized from commercially available compound **46** by reported method [21**]**. Compound **49** was synthesized in the presence of lithium perclorate in ACN. Compound **49** was then cyclised in the presence of CDI to yield compound **50**. The compound **51** was obtained by debenzylation by palladium hydroxide. The acetylation was carried out with Ac_2O in the presence of THF and TEA to yield compound **52**.

<< Scheme 1 >>

<< Scheme 2 >>

<< Scheme 3 >>

2.2 Biological evalutions

The present investigation explored the effect of replacement of oxidatively-labile morpholine ring in **1** with bioisosteric azaspiro substructure (Figure 2). Further, the effect of such structural

modification on the antibacterial and antitubercular activities of **1** was investigated. The azaspiro analogue of **1** (compound **18**) retained the antibacterial activity profile of **1** except complete abrogation of anti-*E. coli* activity (Table **1** and Table S1, Supporting Information section). Similarly, **18** retained the antitubercular activity of **1** (Table 2, Table S1, and Supporting Information). Intrigued by these observations, the authors systematically studied the SAR of compound **18** at the 5-methylamino substituent on the oxazolidinone ring. A total of three series (amides, thioamides and sulphonamides) were designed, synthesized and tested. The protocols and results of primary screening (antibacterial and antitubercular) are given in Supporting Information section (Table S1). Tables **1** and **2** list MIC and IC_{50} (µg/mL) values of the title compound which exhibited >75 % inhibition at 3 µg/mL concentration during primary screening [22].

Careful inspection of the preliminary screening data showed the following activity trends. (Some of the less active compounds and their details are given in the Supporting Information section - Table 1S.) Compound **17** is an azaspiro analogue of compound **1** and was as a precursor to compound **18**. Compound **17** retained reasonable activity against *E. coli* and *S. aureus* but lost its activity against other strains. Acetylating compound **17** resulted in compound **18,** an exact azaspiro analogue of compound **1**. Compound **18** exhibited activity similar to **1** in all tested strains except *E. coli*. Since Linezolid is an acetyl derivative, other acetyl compounds were synthesized and tested (**18, 19, 20, 21, 22, 35, 36** and **37**). Generally it was observed that acetylation of the free amine in compound **17** with bulky substituents (**35**, **36**) led to complete loss of activity. 2-Acetoxyacetyl substitution (**21**) or 2-methoxyacetyl substitution (**37**) led to significant loss of both antibacterial and antitubercular activity. Upon acetylation with aromatic substituents led to mixed results. Isoxazole substitution (**22**) led to encouraging activity trends across various tested strains but pyridine (**19**) and CF_3-substituted phenyl (**20**) moieties led to significant loss of activity across various strains. Compound **19** retained good activity against *E. coli*.

Replacing the amide bond in compound **1** with a sulphonamide linkage led to compounds **23**, **24**, **38**, **39** and **40**. This was an unfavourable transformation where all compounds exhibited loss of activity except compound **23** which retained antibacterial activity.

Different urea and thiourea derivatives of compound **17** were also evaluated. The urea derivatives such as **25-27** and **41** exhibited loss of antibacterial activity and complete abrogation of antitubercular activity. Compounds **28-34** and **42** (thiourea derivatives) were retained antibacterial activity against two or more strains with complete abolition of antitubercular activity. Compound **34** with polar 4-nitrophenyl substituent completely found to lose both the activities. To compare urea and thiourea, two compounds (**41**, **42**) were synthesized**;** unfortunately both the compounds were found to be inactive in antibacterial and antitubercular assays. Tables **1** and **2** listed the antibacterial and antitubercular activities (MIC and IC_{50} values (μg/mL)) of the compounds showing >75% inhibition at 3 μg/mL concentration [23]. Compound **22** was found to be the best in the series with slightly reduced antitubercular activity compared to **1**.

Majority of the compounds showed mixed antibacterial (Gram-positive versus Gram-negative) profiles. Compound **18** exhibited slightly reduced activity in comparison to **1**. A minor change (morpholine in **1** to azaspiro morpholine in **18**) led to marginal reduction in the activity. This may be due to the linear conformation adopted by azaspiro morpholine as opposed to the chair conformation of the morpholine ring. It may affect the interaction with its molecular target with direct effect on the potency. Overall, the present study attempted replacement of oxidatively labile morpholine ring in **1** with its azaspiro analogue and subsequent SAR investigations.

Among the compounds synthesized using Scheme 3, only compound **51** showed good activities against *E. coli* while no activity was shown against other bacterial strains. Unfortunately, it was also not exhibited antitubercular activity. Compound **52**, central spiro analogue of linezolid (**1**), was inactive in both antitubercular and antibacterial assays.

<<<<Table 1>>>>

<<<Table 2>>>

In silico ADMET predictions

Nowadays absorption, distribution, metabolism, excretion and toxicity (ADMET) data has got significant importance in the drug discovery process since several studies have indicated that approximately 40% of drug candidates fail in clinical trials owing to poor pharmacokinetics and toxicity properties. These late-stage failures contribute significantly to the cost of new drug

discovery projects. Therefore, ability to detect problematic candidates in the early stage of drug discovery significantly reduces the risk of late-stage attrition in optimizing the screening and the cost of new drug discovery campaign and focuses the lead optimization efforts to enhance the desired ADMET profiles. Also considering the cost involved in the experiments to obtain the ADMET data, the *in silico* approach provides a cost-effective alternative to filter and optimize the leads in the early phase of drug discovery. With this objective, *in silico* ADMET predictions were performed on the novel azaspiro analogs investigated in the present study to gauge their pharmacokinetic, safety and drug-likeness profile using the QikProp module incorporated in Small-Molecule Drug Discovery Suite (QikProp, version 4.6, Schrödinger, LLC, New York, NY, 2015). The prediction data is summarized in Table 3. In this study, we have evaluated molecular weight, number of hydrogen bond acceptor and donor functional groups, logarithm of partition coefficient (Log *P*), aqueous solubility, brain/blood partition coefficient, binding to human serum albumin and % human oral absorption.

Oral bioavailability is often a desirable property for a drug candidate. The molecules investigated herein have shown moderate-to-high oral bioavailability as reflected from the % human oral absorption data with very low susceptibility to acid hydrolysis in the stomach. The partition coefficient values (logPo/w) and prediction of binding to human serum albumin (logKhsa) suggests that these molecules are hydrophilic in nature which is further confirmed by the logS values suggesting that these molecules have adequate aqueous solubility. Drugs that act at the central nervous system are expected to cross the blood-brain barrier (BBB) in order to reach their site of action while those with peripheral site of actions are expected to have no brain penetration to avoid related side effects. Low values predicted for brain/blood partition coefficient signify that these molecules will have very low propensity to cross the BBB, thereby eliminating the chances of CNS-related toxicity. All of these azaspiro analogs have favorable drug-like properties and are potentially interesting for future lead optimization.

<<<Table 3>>>

3. Conclusions

The present study successfully attempted the replacement of oxidatively-labile morpholine ring in a leading antibacterial drug, linezolid (**1)**, with its azapiro analogue. This is an example of the

successful use of the azaspiro and related substructures in the medicinal chemistry of therapeutically useful compounds. Novel central azaspiro ring containing analogue of linezolid was synthesized (compound **52**). Further SAR investigations led to identification of several mixed (Gram-positive and Gram-negative) antibacterial and antitubercular agents. The ADMET predictions for these molecules were also found to be within tolerance limit qualifying them for development of oral drug candidates. This study provides novel molecules for further exploration in our quest for novel antibacterial and antitubercular agents. In our opinion, this is a valuable investigation with significant impact on antibacterial and antitubercular drug development field.

4. Experimental Section

4.1 General.

Reagents and solvents were obtained from commercial suppliers, Sigma-Aldrich, USA and were used as received unless otherwise indicated. Solvents were dried, wherever necessary, according to standard procedures. All reactions were performed under an inert atmosphere (N2) unless otherwise indicated. Analytical silica gel 60 F_{254}-coated TLC plates were purchased from EMD Chemicals, USA, and were visualized with UV light. The ^{1}H-NMR spectra were routinely obtained with a Varian Mercury Plus 300 MHz NMR and 400 MHz (Agilent Technologies, Santa Clara, USA) and TMS was used as an internal standard. LC-MS spectra were recorded on 6110 AA Series Quadrupole LC/MS system (Agilent Technologies, Santa Clara, USA). Analytical HPLC studies were carried out with a Kromasil® 100-5C_{18} column (150 mm X 4.6 mm) on PerkinElmer Series 200 HPLC system with auto sampler and PDA detector (PerkinElmer, Inc., Waltham, USA). Melting points were recorded using a Veego (VMP)-D capillary melting point apparatus (Veego Instruments Corp. Mumbai, India) and were uncorrected.

4.2 Synthesis

4.2.1 Synthesis of 6-tosyl-2-oxa-6-azaspiro[3.3]heptane (9): To a solution of benzenesulphonamide (**8**) (5.81 g, 36.9 mmol) in 500 mL of EtOH, 3-bromo-2,2-bis(bromomethyl)propan-1-ol (**9**) (10 g, 30.8 mmol), KOH (5.53 g, 99 mmol) was added and heated to reflux at 90 °C for 48 hrs. The solvent was evaporated and 100 mL of 1M KOH was added and the resultant white suspension was stirred for another 2 hrs at RT. The mixture was

filtered and the residue was rinsed with water until the filtrate was neutral. The filter cake was dried under high vacuum to give crude product (4.5 g). Yield: 57.7 %; off-white solid; ^{1}H-NMR (300 MHz, DMSO-*d6*) δ (ppm): 7.69 (d, *J* = 8Hz, 2H, Ar-H), 7.49 (d, *J* = 8.1Hz, 2H, Ar-H), 4.42 (s, 4H, $2CH_2$, spiro morpholine), 3.84 (s, 4H, $2CH_2$, spiro morpholine), 2.42 (s, 3H, CH_3); LC-MS (ESI +ve) *m/z* 254 $[M+H]^+$; HPLC purity: 99.79 %

4.2.2 Synthesis of of 2-oxa-6-azaspiro[3.3]heptane oxalate (10): To a solution of **9** (7.5 g, 29.6 mmol) in 500 mL MeOH, magnesium turnings (5.04 g, 207 mmol) were added and reaction mass was sonicated for 1 hr. Then solvent was evaporated and 1 L of diethyl ether was added along with sodium sulphate decahydrate (4.21 g, 29.6 mmol) and stirred for 1 hr at RT. The reaction mixture was filtered through celite and dried over anhyd. Na2SO4 and then oxalic acid (1.33 g, 14.80 mmol) dissolved in 2 mL EtOH added and stirred for 5 min. to get oxalate salt of amine (10) (1.5 g). Yield: 51.1% ; off-white solid; ^{1}H-NMR (300 MHz, DMSO-*d6*) δ (ppm): 4.65 (s, 4H, $2CH_2$, spiro morpholine), 4.16 (s, 4H, $2CH_2$, spiro morpholine); LC-MS (ESI +ve) *m/z* 100.0 $[M+H]^+$.

4.2.3 Synthesis of 6-(2-fluoro-4-nitrophenyl)-2-oxa-6-azaspiro[3.3] heptane (12): To a solution of **11** (6.99 mL, 62.9 mmol) in 20 mL of ACN, were added 2-oxa-6-azaspiro[3.3]heptane (1) (4 g, 27.6 mmol) and DIPEA (32.9 mL, 189 mmol) and the reaction mixture heated to 90 °C overnight. The cooled to 0 - 5 °C and filtered to get the crude product (8 g). Yield: 53.4 %; yellow solid; ^{1}H-NMR (300 MHz, DMSO-d6) δ (ppm): 7.95 (d, *J* = 9Hz, 2H, Ar-H), 6.59 (t, *J* = 9Hz, 1H, Ar-H), 4.7 (s, 4H, $2CH_2$, spiro morpholine), 4.35 (s, 4H, $2CH_2$, spiro morpholine); ^{13}C-NMR (50 MHz, DMSO-*d6*) δ: 150.98, 144.20, 135.41, 122.01(2C), 111.94, 62.64(2C), 56.51(2C), 42.10; LC-MS (ESI +ve) *m/z* 239.1 $[M+H]^+$; HPLC Purity: 98.88%.

4.2.4 Synthesis of 3-fluoro-4-(2-oxa-6-azaspiro[3.3]heptan-6-yl) aniline (13). To a solution of **12** (5 g, 20.99 mmol) in 25 mL EtOH, Pd-C (2.23 g, 20.99 mmol) was added and reaction mass was stirred for 4 hrs at 65-80 PSI at RT under H2 atmosphere Parr® shaker. The reaction was monitored by TLC. After completion, the contents of the flask were filtered through celite and concerntrated to get pure (13) (4 g). Yield: 92 %; brown solid; ^{1}H-NMR (300 MHz, DMSO-*d6*) δ (ppm): 6.31-6.23 (m, 3H, Ar-H), 4.75 (s, 4H, $2CH_2$, spiro morpholine), 3.81 (s, 4H, $2CH_2$, spiro morpholine); ^{13}C-NMR (50 MHz, DMSO-*d6*) δ: 155.18, 142.50, 129.60, 115.69, 109.85,

102.28, 79.79(2C), 62.31(2C), 40.76; LC-MS (ESI +ve) m/z 209.1 [M+H]+; HPLC Purity: 99.73 %.

4.2.5 Synthesis of (R)-2-(3-((3-fluoro-4-(2-oxa-6-azaspiro[3.3]heptan-6-yl)phenyl)amino)-2-hydroxypropyl) isoindoline-1,3-dione (15): To a solution of **13** (2.95 g, 14.17 mmol), (S)2-(oxiran-2-ylmethyl)isoindoline-1,3-dione (14) (5.4 g, 26.6 mmol) in 10 mL DMF, DIPEA 4.7 mL was added and reaction mass was heated at 120 °C overnight. The solvent was evaporated, water was added and the mixture was extracted with DCM. It was purified using flash chromatography (ethyl acetae: pet ether 40:60) to yield pure product (4 g). Yield: 54.9 %; brown solid; m.p. 128-130 °C; $[\alpha]^{20}_{D}$ = -16^{o} (c = 0.1 , $CHCl_3$); ^{1}H-NMR (300 MHz, DMSO-*d6*) δ (ppm): 7.85 (d, *J* = 3.6, 4H, Pthalimide), 6.42-6.34 (m, 3H, Ar- H), 5.24-5.22 (m, 1H, CH-OH), 5.14 (d, *J* = 5.1Hz, 1H, NH), 4.68 (s, 4H, $2CH_2$, spiro morpholine), 4.0-3.97 (m, 1H, OH), 3.8 (s, 4H, $2CH_2$, spiro morpholine), 3.63-3.58 (m, 2H, CH_2-pthalimide), 3.05-3.02 (m, 1H, NHCH-HOH), 2.94-2.92 (m, 1H, NHCH-HOH); ^{13}C-NMR (50 MHz, DMSO-*d6*) δ: 168.05 (2C), 154.13, 143.13, 134.20 (2C), 131.79 (2C), 129.52, 122.87(2C), 115, 108, 100, 79.79(2C), 68.57(2C), 62.33(2C), 48.03, 42.39: LC-MS (ESI +ve) *m/z* 412.1 $[M+H]^+$; HRMS (ESI +ve) *m/z* $[M+H]^+$ calcd for $C_{22}H_{22}FN_3O_4$: 412.1667, found 412.1672; HPLC Purity: 98.73 %.

4.2.6 Synthesis of (S)-2-((3-(3-fluoro-4-(2-oxa-6-azaspiro[3.3]heptan-6-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)isoindoline-1,3-dione (16). A reaction mixture containing **15** (4 g, 9.72 mmol), CDI (2.365 g, 14.58 mmol), DMAP (0.594 g, 4.86 mmol) in 10 mL THF was heated at 60 °C overnight. After completion, the reaction mass was concentrated and water was added followed by extraction in DCM. The organic layers were dried over sodium sulphate and purification was done with the help of flash chromatography in ethyl acatete and pet ether (40:60) to get pure product (2.6 g). Yield: 61.1 %; yellow soild; m.p. 203-205 °C; $[\alpha]^{20}_{D}$ = -84^{o} (c = 0.1 , $CHCl_3$); ^{1}H-NMR (300 MHz, DMSO-*d6*) δ (ppm): 7.92-7.84 (m, 4H, Ar-H), 7.32 (d, *J* = 15 Hz, 1H, Ar- H), 7.0 (d, *J* = 8.4Hz, 1H, Ar- H), 6.60 (t, *J* = 9.3Hz, 1H, Ar- H), 4.8-4.69 (m, 1H, CH oxazolidone ring), 4.69 (s, 4H, $2CH_2$ spiro morpholine), 4.13 (t, *J* = 9Hz, 1H, CH_2 of oxazolidone ring), 4.0 (s, 4H, $2CH_2$ of spiro morpholine), 3.95-3.92 (m, 1H, CH_2 of oxazolidone ring), 3.91-3.89 (m, 2H, CH_2- pthalimide); ^{13}C-NMR (100 MHz, DMSO-d6) δ: 167.77(2C), 153.81, 134.60(2C), 131.48(2C), 123.23 (4C), 114.84 (2), 107.12 (2), 79.71 (3C), 69.93,

62.27(2C), 48.07, 40.47; LC-MS (ESI +ve) *m/z* 438.1 $[M+H]^+$; HRMS (ESI +ve): *m/z* $[M+H]^+$ calcd for $C_{22}H_{22}FN_3O_4$: 438.1460, found 438.1465; HPLC Purity: 92.07%.

4.2.7 Synthesis of (S)-5-(aminomethyl)-3-(3-fluoro-4-(2-oxa-6-azaspiro[3.3]heptan-6-yl)phenyl)oxazolidin-2-one (17): To a solution of **16** (3 g, 6.86 mmol) in 10 mL EtOH, hydrazine (1.722 mL, 54.9 mmol) was added and the reaction mixture refluxed for 2 hrs. The solvent was evaporated and the residue washed with water and purified on flash chromatography using DCM and MeOH (95:5) to get pure product (5 g). Yield: 71.2%; off-white solid; m.p. 118-120 °C; $[\alpha]^{20}_D$ = -96° (c = 0.1 , $CHCl_3$); ^{1}H-NMR (300 MHz, DMSO-*d6*) δ (ppm): 7.42 (d, *J* = 15Hz, 1H, Ar- H), 7.14 (d, *J* = 9Hz, 1H, Ar-H), 6.57 (d, *J* = 9.9Hz, 1H, Ar- H), 4.7 (s, 4H, $2CH_2$ spiro morpholine), 4.56- 4.54 (m, 1H, CH of oxazolidone), 3.98-3.95 (s, 5H, $2CH_2$ spiro morpholine), 3.81 (t, *J* = 7.5Hz, 1H, CH_2 of oxazolidone ring H), 2.81-2.77 (m, 2H,CH_2 attached to amine), 1.91-1.89 (m, 2H); LC-MS (ESI +ve) *m/z* 308.1 $[M+H]^+$; HRMS (ESI +ve) *m/z* $[M+H]^+$ calcd for $C_{15}H_{18}FN_3O_3$: 308.1405, found 308.1395; HPLC Purity: 98.49 %.

4.2.8 Synthesis of (S)-N-((3-(3-fluoro-4-(2-oxa-6-azaspiro[3.3]heptan-6-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)acetamide (18): To a solution of **17** (100 mg, 0.325 mmol) in 5 mL DCM, TEA (181 µL, 1.302 mmol) was added and the reaction mixture cooled to 0 °C, acetic anhydride (36.8 µL, 0.390 mmol) was added which was previously cooled to 0 °C and then stirred at RT for 1 hr. After completion, water was added and the reaction mixture extracted with DCM. The combined organic layers were which was dried over sodium sulfate and the solvent evaporated followed by the purification of the residue by flash chromatography using EtOAc and pet ether (40:60) to get pure product (90 mg). Yield: 79%; off-white solid; m.p. 178-180 °C; $[\alpha]^{20}_D$ = - 8° (c = 0.1, $CHCl_3$); ^{1}H-NMR (300 MHz, DMSO-*d6*) δ (ppm): 8.25 (t, *J* = 3.6Hz, 1H, amide), 7.38 (d, *J* = 7.8Hz, 1H, Ar -H), 7.10 (d, *J* = 5.1Hz, 1H, Ar-H), 6.60 (d, *J* = 6Hz, 1H, Ar-H), 4.70-4.65 (m, 5H, CH_2 spiro morpholine and CH_2 oxazolidone ring), 4.05-4.02 (m, 5H, CH_2 spiro morpholine and CH_2 oxazolidone ring), 3.67-3.65 (m, 1H, CH_2 oxazolidone ring), 3.39 (t, *J* = 3Hz, 2H, CH_2 attach to amide), 1.83 (s, 3H, methyl); ^{13}C-NMR (100 MHz, DMSO-*d6)* δ: 169, 154, 152, 135, 130, (2)114, 106, (2C)79, 71, (2C) 62, 47, 40, 38, 22; LC-MS (ESI +ve) *m/z* 350.1 $[M+H]^+$; HRMS (ESI +ve) *m/z* $[M+H]^+$ calcd for: $C_{17}H_{20}FN_3O_4$; 350.1511, found 350.1482; HPLC Purity: 99.79 %.

4.2.9 Compounds 19, 20, 21, 22, 35, 36, 37 were synthesized using similar procedure for 18.

4.2.10 Synthesis of (S)-N-((3-(3-fluoro-4-(2-oxa-6-azaspiro[3.3]heptan-6-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)nicotinamide (19): To a solution of **17** (100 mg, 0.325 mmol) in 5 mL of DCM, TEA (181 µL, 1.302 mmol) added and reaction was cooled to 0 °C, nicotinoyl chloride (87 mg, 0.488 mmol) was added and stirred for 2 hrs at RT. Work up was done similar to compound (18) and purified by flash chromatography in DCM and MeOH (95:5) to get pure product (76 mg). Yield: 56.6%; off-white solid; m.p. 176-178 °C; ^{1}H-NMR (300 MHz, DMSO-*d6*) δ (ppm): 9.0 (m, 2H), 8.72 (d, *J* = 4.5Hz, 1H), 8.18 (d, *J* = 8.1Hz, 1H), 7.53 (m, 1H), 7.39 (m, 1H), 7.12 (d, *J* = 8.4Hz, 1H), 6.60 (t, *J* = 9.3Hz, 1H), 4.82-4.80 (m, 1H), 4.7 (s, 4H), 4.1 (t, *J* = 9Hz, 1H), 4.0 (s, 4H), 3.79-3.80 (m, 1H), 3.64-3.62 (m, 2H); ^{13}C-NMR (50 MHz, $CDCl_3$) δ : 166.68, 154.70, 152.22, 148.33(2C), 135.45(2C), 129.61(2C), 123.47(2C), 114.60, 107.45, 80.96(2C), 71.87, 62.73(2C), 48.12, 42.68, 39.78; LC-MS (ESI +ve) *m/z* 413.2 $[M+H]^+$; HRMS (ESI +ve) *m/z* $[M+H]^+$ calcd for: $C_{21}H_{21}FN_4O_4$; 413.1620, found 413.1614; HPLC Purity: 99.29 %.

4.2.11 Synthesis of (S)-N-((3-(3-fluoro-4-(2-oxa-6-azaspiro[3.3]heptan-6-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)-4-(trifluoromethyl)benzamide (20): To a solution of **17** (100 mg, 0.325 mmol) in 5 mL of DCM, TEA (181 µL, 1.302 mmol) added and reaction was cooled to 0 °C, 4-(trifluoromethyl)benzoyl chloride (88 mg, 0.423 mmol) was added and stirred for 2 hrs at RT. Work up was done similar to compound (18) and purified by flash chromatography in DCM and MeOH (95:5) to get pure product (70 mg). Yield: 44.88 %; off-white; m.p. 209-210 °C; ^{1}H-NMR (300 MHz, DMSO-*d6*) δ (ppm): 9.04-9.02 (m, 1H), 8.04 (d, *J* = 8.1Hz, 2H), 8.18 (d, *J* = 8.1Hz, 2H), 7.39-7.34 (m, 1H), 7.12-7.10 (m, 1H), 6.60 (t, *J* = 9.3Hz, 1H), 4.84-4.80 (m, 1H), 4.7 (s, 4H), 4.1 (t, *J* = 9Hz, 1H), 4.0 (s, 4H), 3.80 - 3.77 (m, 1H), 3.64-3.63 (m, 2H); ^{13}C-NMR (50 MHz, $CDCl_3$): δ 166.54, 153.96, 136.79, 129.40, 127.56(5C), 4.67(2C),113.84(2C), 107.20, 106.73, 80.29(2C), 71.02, 62.17(2C), 47.64, 42.26, 39.50; LC-MS (ESI +ve) *m/z* 480.2 $[M+H]^+$; HRMS (ESI +ve) *m/z* $[M+H]^+$ calcd for: $C_{23}H_{21}FN_3O_4$; 480.1541, found 480.1579; HPLC Purity: 97.77 %.

4.2.12 Synthesis of (S)-2-(((3-(3-fluoro-4-(2-oxa-6-azaspiro[3.3]heptan-6-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)amino)-2-oxoethyl acetate (21): To a solution of **17** (100 mg, 0.325 mmol) in 5 mL of DCM, TEA (181 µL, 1.302 mmol) was added and the reaction mixture was cooled to 0 °C, 2-chloro-2-oxoethyl acetate (66.6 mg, 0.488 mmol) was added and stirred for 2

hrs at RT. Work up was done similar to compound (**18**) followed by purification using flash chromatography in DCM and MeOH (95:5) to get pure product (69 mg). Yield: 52.1 %; off-white solid; m.p. 103-105 °C; ^{1}H-NMR (300 MHz, DMSO-d6) δ (ppm): 8.40 (s, 1H), 7.39 (d, *J* = 14.7Hz, 1H), 7.11 (d, *J* = 8.7Hz, 1H), 6.61 (t, *J* = 9.Hz, 1H), 4.70 (s, 4H), 4.46 (s, 2H), 4.22-4.20 (m, 2H), 4.02 (s, 4H), 3.69-3.67 (m, 1H), 3.44-3.40 (m, 2H), 2.06 (s, 3H); HRMS (ESI +ve) *m/z* $[M+H]^+$ calcd for $C_{19}H_{22}FN_3O_6$; 408.1565, found 408.1549; HPLC Purity: 98.31%.

4.2.13 Synthesis of (S)-N-((3-(3-fluoro-4-(2-oxa-6-azaspiro[3.3]heptan-6-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)isoxazole-5-carboxamide (22): To a solution of **17** (100 mg, 0.325 mmol) in 5 mL of DCM, TEA (181 µL, 1.302 mmol) added and reaction was cooled to 0 °C, isoxazole-5-carbonyl chloride (47.2 µL, 0.488 mmol) was added and stirred for 2 hrs at RT. Work up was done similar to compound (18) and purified by flash chromatography in DCM and MeOH (95:5) to get pure product (80 mg). Yield: 61.1 %; pale yellow solid; m.p. 164-165 °C; ^{1}H-NMR (400 MHz, DMSO-*d6)* δ (ppm): 9.30 (t, *J* = 8Hz, 1H), 8.75 (d, *J* = 1.92Hz, 1H), 7.37 (dd, *J* = 2.4Hz, 1.64 Hz, 1H), 7.10 (d, *J* = 1.6Hz, 1H), 7.08 (d, *J* = 2.4Hz, 1H), 6.59 (t, *J* = 8.80Hz, 1H) 4.81-4.49 (m, 1H), 4.70 (s, 4H), 4.12 (t, *J* = 9Hz, 1H), 4.02 (s, 4H), 3.80-3.76 (m, 1H), 3.61-3.58 (m, 2H); HRMS (ESI +ve) *m/z* $[M+H]^+$ calcd for $C_{19}H_{19}FN_4O_5$; 403.1412, found 403.1450; HPLC Purity: 92.36 %.

4.2.14 Synthesis of (R)-5-chloro-N-((3-(3-fluoro-4-(2-oxa-6-azaspiro[3.3]heptan-6-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)thiophene-2-sulfonamide (23): To a solution of **17** (100 mg, 0.325 mmol) in 5 mL of DCM, TEA (181 µL, 1.302 mmol) added and reaction was cooled to 0 °C, 5-chlorothiophene-2-sulfonyl chloride (106 mg, 0.488 mmol) was added and stirred for 2 hrs at RT. Then water was added and extracted with DCM which was dried over sodium sulfate. The DCM layer was concentrated and purified by flash chromatography in DCM and MeOH (95:5) to get pure product (85 mg). Yield: 53.5 %; off-white solid; m.p. 98-100 °C; ^{1}H-NMR (300 MHz, DMSO-*d6*) δ (ppm): 8.50 (s, 1H), 7.52 (d, *J* = 4.2Hz, 1H), 7.39 (d, *J* = 2.1Hz , 1H), 7.34 (d, *J* = 2.1Hz, 1H), 7.09-7.06 (m, 1H), 6.61 (t, *J* = 10.2Hz, 1H), 4.70 (s, 5H), 4.02 (s, 5H), 3.71-3.66 (m, 1H), 3.22-3.19 (m, 2H); ^{13}C-NMR (100 MHz, DMSO-*d6*) δ: 153.91, 152.41, 139.48, 135.54, 134.54, 131.58, 130.04, 127.99, 114.74(2C), 106.91, 79.70(2C), 70.88, 62.25(2C), 47.11, 45.36, 40.12; LC-MS (ESI +ve) *m/z* 488.1 $[M+H]^+$; HRMS (ESI +ve) *m/z* $[M+H]^+$calcd for: $C_{19}H_{19}ClFN_3O_5S_2$; 488.0511, found 488.0501; HPLC Purity: 99.29 %.

4.2.15 Compounds 24, 38, 39, 40 were synthesized similar to 23

4.2.16 Synthesis of (R)-N-((3-(3-fluoro-4-(2-oxa-6-azaspiro[3.3]heptan-6-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)-3-(trifluoromethyl)benzenesulfonamide (24): To a solution of **17** (100 mg, 0.325 mmol) in 5 mL of DCM, TEA (181 µL, 1.302 mmol) added and reaction was cooled to 0 °C, 3-(trifluoromethyl)benzene-1-sulfonyl chloride (79 µL, 0.488 mmol was added and stirred for 2 hrs at RT. Work up and purification was done similar to **23** to get pure product (76 mg). Yield: 45.32%; white solid; m.p. 98-101 °C; ^{1}H-NMR (300 MHz, DMSO-*d6*) δ (ppm): 8.44 (d, *J* = 8.1Hz, 1H), 8.35 (s, 1H), 8.25 (d, *J* = 7.8Hz, 1H), 7.99 (t, *J* = 8.1Hz, 2H), 7.41-7.36 (m, 1H), 7.12-7.09 (m, 1H), 6.63 (t, *J* = 9.3Hz, 1H), 4.96-4.92 (m, 1H), 4.7 (s, 4H), 4.45-4.36 (m, 1H), 4.15-4.09 (m, 2H), 4.03 (s, 4H), 3.74-3.72 (m, 1H); LC-MS (ESI +ve) *m/z* 516.1 $[M+H]^+$; HRMS (ESI^+) *m/z* $[M+H]^+$ calcd for: $C_{22}H_{21}F_4N_3O_5S$; 515.1133, found 515.1109; HPLC Purity: 98.31 %.

4.2.17 Synthesis of (S)-1-((3-(3-fluoro-4-(2-oxa-6-azaspiro[3.3]heptan-6-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)-3-(4-fluorophenyl)urea (25): To a solution of **17** (100 mg, 0.325 mmol) in 5 mL of DCM, TEA (181 µL, 1.302 mmol) added and reaction was cooled to 0 °C, 4-flurohenyl isocynate (54.9 µL, 0.488 mmol) was added and stirred for 2 hrs at RT. Then water was added and extracted with DCM which was dried over sodium sulfate. The DCM layer was concentrated and purified by flash chromatography in DCM and MeOH (95:5) to get pure product (67 mg). Yield: 46.3%; off-white solid; m.p. 215-217°C; ^{1}H-NMR (400 MHz, DMSO-d6) δ (ppm): 8.60 (s, 1H), 7.38-7.34 (m , 3H), 7.11-7.02 (m, 3H), 6.58 (t, *J* = 9.48Hz, 1H), 6.47-6.44 (t, *J* = 5.88, 1H) 4.74-4.735 (m, 1H), 4.69 (s, 4H), 4.08-4.065 (t, *J* = 9Hz, 1H), 4.01 (s, 4H), 3.72 (m, 1H), 3.45-3.43 (t, *J* = 8Hz, 2H); LC-MS (ESI +ve) *m/z* 445.2 $[M+H]^+$; HPLC Purity: 99.55 %.

4.2.18 Compounds 26, 27 and 41 were synthesized similar to 25.

4.2.19 Synthesis of (S)-1-((3-(3-fluoro-4-(2-oxa-6-azaspiro[3.3]heptan-6-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)-3-(3-(trifluoromethyl)phenyl)urea (26): 17 (100 mg, 0.325 mmol) in 5 mL of DCM, TEA (181 µL, 1.302 mmol) added and reaction was cooled to 0 °C, 1-isocyanato-3-(trifluoromethyl)benzene (91 mg, 0.488 mmol) was added and stirred for 2 hrs at RT. Work up and purification was done similar to (25) to get pure product (75 mg). Yield: 46.6 %; white solid; m.p. 87-90 °C; ^{1}H-NMR (300 MHz, DMSO-*d6*) δ (ppm): 8.9 (s, 1H), 7.95 (s,

1H), 7.47 (m, 1H), 7.37-7.35 (m, 2H), 7.25 (d, *J* = 6.3Hz, 1H), 7.13 (d, *J* = 8.4, 1H), 6.62-6.59 (m, 2H), 4.91-4.90 (m, 1H), 4.7 (s, 4H), 4.11-4.08 (m, 1H), 4.0 (s, 4H), 3.75 (t, *J* = 6.3Hz, 1H), 3.47-3.46 (m, 2H); ^{13}C-NMR (100 MHz, DMSO-*d6*) δ: 155.25, 152.42, 150.04, 141.01, 135.51, 130.14, 129.76, 129.58, 129.26, 121.25, 117.50, 114.70, 114.60, 113.65, 106.94, 79.70(2C), 71.77, 62.24(2C), 47.90, 41.94, 39.91; HRMS (ESI$^+$) *m/z* $[M+H]^+$ calcd for: $C_{23}H_{22}F_4N_4O_4$.; 495.4650, found 495.1629; HPLC Purity: 96.29 %.

4.2.20 Synthesis of (S)-1-((3-(3-fluoro-4-(2-oxa-6-azaspiro[3.3]heptan-6-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)-3-(2-fluorophenyl)urea (27): To a solution of **17** (100 mg, 0.325 mmol) in 5 mL of DCM, TEA (181 µL, 1.302 mmol) added and reaction was cooled to 0 °C, 1-fluoro-2-isocyanatobenzene (66 mg, 0.4817. mmol) was added and stirred for 2 hrs at RT. Work up and purification was done similar to (25) to get pure product (80 mg). Yield: 55.3 %; white solid; m.p. 1483-205 °C; ^{1}H-NMR (400 MHz, DMSO-*d6*) δ (ppm): 8.43 (s, 1H), 8.0 (t, *J* = 1.6Hz , 1H), 7.40-7.38 (m, 1H), 7.17-6.91 (m, 5H), 6.91 (t, *J* = 8.80Hz , 1H), 4.74 - 4.73 (m, 1H), 4.70 (s, 4H), 4.09 (t, *J* = 9Hz, 1H), 4.01 (s, 4H), 3.80-3.76 (m, 1H), 3.48-3.32 (m, 2H); ^{13}C-NMR (100 MHz, DMSO-*d6*) δ: 155.07, 154.113, 152.82, 150.42, 135.53, 130.12, 128.03, 124.35, 121.81, 120.20, 114.88, 114.69, 114.64, 106.97, 79.69(2C), 71.86, 62.24(2C), 47.33, 41.84, 40.13; HRMS (ESI$^+$) *m/z* $[M-H]^-$ calcd for $C_{22}H_{22}F_2N_4O_4$; 443.1525, found 443.1561; HPLC Purity: 98.66 %

4.2.21 Synthesis of (S)-1-(4-cyanophenyl)-3-((3-(3-fluoro-4-(2-oxa-6-azaspiro[3.3]heptan-6-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)thiourea (28): To a solution of **17** (100 mg, 0.325 mmol) in 5 mL of DCM, TEA (181 µL, 1.302 mmol) added and reaction was cooled to 0 °C, 4-isothiocyanatobenzonitrile (78 mg, 0.488 mmol) was added and stirred for 2 hrs at RT. Then water was added and extracted with DCM which was dried over sodium sulfate. The DCM layer was concentrated and purified by flash chromatography in DCM and MeOH (95:5) to get pure product (70 mg). Yield: 47.9 %; off-white solid; m.p. 196-198 °C; ^{1}H-NMR (300 MHz, DMSO-*d6*) δ (ppm): 10.12 (s, 1H), 8.4 (s, 1H), 7.74 (s, 4H), 7.42 (d, *J* = 14.4Hz, 1H), 7.14 (d, 1H), 6.61 (t, *J* = 9H, 1H), 4.92-4.90 (m, 1H), 4.7 (s, 4H), 4.01 (s, 4H), 3.92-3.82 (m, 1H), 3.8 (s, 1H), 3.17 (d, *J* = 5.1Hz, 1H), 2.7 (d, *J* = 12.36Hz, 1H); ^{13}C-NMR (100 MHz, DMSO- *d*6) δ : 180.97, 154.03, 152.41, 143.87, 135.56, 132.77(2C), 130.06, 121.56, 119.03, 114.66(2C), 107.2(2C), 105.10, 79.71(2C), 70.92, 62.27(2C), 47.40, 46.38, 40.12; HRMS (ESI$^+$) m/z $[M+H]^+$ calcd for: $C_{23}H_{22}FN_5O_3S$; 468.1500, found 468.1474; HPLC Purity: 98.42 %.

4.2.22 Compounds from 29 to 33 and 42 are synthesized similar to 28

4.2.23 Synthesis of (S)-1-((3-(3-fluoro-4-(2-oxa-6-azaspiro[3.3]heptan-6-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)-3-(3-(trifluoromethyl)phenyl)thiourea (29): To a solution of **17** (100 mg, 0.325 mmol) in 5 mL of DCM, TEA (181 µL, 1.302 mmol) added and reaction was cooled to 0 °C, 1-isothiocyanato-3-(trifluoromethyl)benzene (99 mg, 0.488 mmol) was added and stirred for 2 hrs at RT. Work up and purification was done similar to (28) to get pure product (80 mg). Yield: 48.2 %; white solid; m.p. 180-182 °C; ^{1}H-NMR (300 MHz, DMSO-*d*6) δ (ppm): 9.95 (s, 1H), 8.27 (s, 1H), 7.79 (s, 1H), 7.65-7.63 (m, 1H), 7.54-7.37 (m, 3H), 7.14 (d, *J* = 8.7, 1H), 6.59-6.56 (m, 1H), 4.92-4.90 (m, 1H), 4.7 (s, 4H), 4.10-4.09 (m, 1H), 4.0 (s, 4H), 3.90 (s, 1H), 3.82-3.80 (m, 1H), 3.17 (d, *J* = 5.1Hz, 1H); ^{13}C-NMR (100 MHz, DMSO-*d*6) δ:181.47, 154.03, 152.42, 140.18, 135.57, 130.09, 129.61, 129.23, 126.44, 125.38, 122.67, 120.42, 119.03, 114.70, 107.03, 79.71(2C), 71.02, 62.26(2C), 47.40, 46.38, 40.28; HRMS (ESI^{+}) *m/z* [M+H]$^{+}$ calcd for $C_{23}H_{22}F_4N_4O_3S$; 511.1422, found 511.1429; HPLC Purity: 99.71 %.

4.2.24 Synthesis of (S)-1-((3-(3-fluoro-4-(2-oxa-6-azaspiro[3.3]heptan-6-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)-3-isopropylthiourea (30): To a solution of **17** (100 mg, 0.325 mmol) in 5 mL DCM, TEA (181 µL, 1.302 mmol) was added . Reaction mass was cooled to 0 °C, isopropyl Isocyanate (48.3 µL, 0.488 mmol) was added and stirred for 2 hrs at RT. Work up and purification was done similar to (28) to get pure product (70 mg). Yield: 52.7 %; white solid; m.p. 148-150 °C; ^{1}H-NMR (400 MHz, DMSO-d6) δ (ppm): 7.51 (br, s, 2H), 7.39 (dd, *J* = 2.3Hz, 10.98Hz, 1H), 7.11 (dd, *J* = 1.6Hz, 8.6Hz, 1H), 6.59 (t, *J* = 8.9Hz, 1H), 4.80 (m, 1H), 4.70 (s, 4H), 4.20-4.18 (m, 1H), 4.06 (s, 5H), 3.79-3.77 (m, 3H); 1.09-1.05 (m, 6H); ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 154.07, 152.39, 135.56, 130.09, 114.74(2C), 107.07(2C), 79.69(2C), 71.47, 62.25(2C), 47.27, 46.04, 45.07, 39.92, 22.16(2C); HRMS (ESI^{+}) *m/z* [M+H]$^{+}$ calcd for $C_{19}H_{25}FN_4O_3S$; 409.1704, found 409.1752; HPLC Purity: 99.16 %.

4.2.25 Synthesis of (S)-1-((3-(3-fluoro-4-(2-oxa-6-azaspiro[3.3]heptan-6-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)-3-(4-methoxyphenyl)thiourea (31): To a solution of **17** (100 mg, 0.325 mmol) in 5 mL of DCM, TEA (181 µL, 1.302 mmol) added and reaction was cooled to 0 °C, 1-isothiocyanato-4-methoxybenzene (69.9 mg, 0.423 mmol) was added and stirred for 2 hrs at RT. Work up and purification was done similar to (28) to get pure product (79 mg). Yield: 51.4%; white solid; m.p. 176-178 °C; ^{1}H-NMR (400 MHz, DMSO-d6) δ (ppm): 9.50 (s, 1H),

7.74 (s, 1H), 7.38 (dd, *J* = 3.72Hz, 14.64Hz, 1H), 7.17 (d, *J* = 8.84Hz, 2H), 7.11- 7.08 (m, 1H), 6.86 (d, *J* = 8.92Hz, 2H), 6.58-6.53 (m, 1H), 4.87-4.85 (m, 1H), 4.67 (s, 4H), 4.06 (t, *J* = 9Hz, 1H), 3.99 (s, 4H), 3.82-3.80 (m, 3H), 3.70 (s, 3H); ^{13}C-NMR (100 MHz, DMSO-d6) δ : 181.53, 156.68, 154.07, 152.41, 150.03, 135.54, 131.39, 130.12, 125.94, 114.67(2C), 113.30, 92(2C), 79.70(2C), 71.07, 62.26(2C), 55.21, 47.32, 46.53, 40.13; HRMS (ESI$^+$) *m/z* [M-H]- calcd for $C_{23}H_{25}FN_4O_4S$ Na ; 471.1497, found 471.1495; HPLC Purity: 98.95 %.

4.2.26 Synthesis of (S)-1-((3-(3-fluoro-4-(2-oxa-6-azaspiro[3.3]heptan-6-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)-3-(2-fluorophenyl)thiourea (32): To a solution of **17** (100 mg, 0.325 mmol) in 5 mL of DCM, TEA (181 µL, 1.302 mmol) added and reaction was cooled to 0 °C, 1-fluoro-2-isothiocyanatobenzene (74.8 mg, 0.488 mmol) was added and stirred for 2 hrs at RT. Work up and purification was done similar to (28) to get pure product (75 mg). Yield: 50.1 %; off-white solid; m.p. 189-190 °C; ^{1}H-NMR (400 MHz, DMSO-d6) δ (ppm): 9.44 (s, 1H), 8.21 (s, 1H), 7.64 (m, 1H), 7.41 (dd, *J* = 4Hz, 14Hz, 1H), 7.26-7.22 (m, 2H), 7.17-7.16 (m, 2H), 6.60-6.5 (m, 1H), 4.90-4.80 (m , 1H), 4.70 (s, 4H), 4.10 (t, *J* = 9Hz, 1H), 4.01 (s, 4H), 3.87-3.82 (m, 2 H), 3.82-3.78 (m, 1H); HRMS (ESI$^+$) *m/z* [M-H]- calcd for $C_{22}H_{22}F_2N_4O_3S$; 459.1297, found 459.1297; HPLC Purity: 99.87 %.

4.2.27 Synthesis of (S)-1-(tert-butyl)-3-((3-(3-fluoro-4-(2-oxa-6-azaspiro[3.3]heptan-6-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)thiourea (33): To a solution of **17** (100 mg, 0.325 mmol) in 5 mL of DCM, TEA (181 µL, 1.302 mmol) added and reaction mass was cooled to 0 °C, 2-isothiocyanato-2-methylpropane (56.2 mg, 0.488 mmol) was added and stirred for 2 hrs at RT. Work up and purification was done similar to (28) to get pure product (80 mg). Yield: 58.2 %; off-white solid; m.p. 155-158 °C; ^{1}H-NMR (400 MHz, DMSO-*d6*) δ (ppm): 7.53 (t, *J* = 5.56Hz, 1H), 7.39 (dd, *J* = 2.36Hz, 14.64 Hz, 2H), 7.11 (dd, *J* = 2.04Hz, 8.6Hz, 1H), 6.59 (dd, *J* = 8.84Hz, 10.12Hz, 1H), 4.80-4.77 (m , 1H), 4.70 (s, 4H), 4.06-4.01 (m, 5H), 4.02 (s, 9H), 3.80-3.73 (m, 3H); ^{13}C-NMR (50 MHz, DMSO *d6*) δ: 181.93, 154.09, 152.40, 135.57, 130.10, 114.76 (2C), 107.11, 79.70(2C), 71.65, 62.26(2C), 52.26, 47.25, 45.43, 40.13, 28.54(3C); HRMS (ESI +ve) *m/z* [M-H]- calcd for $C_{20}H_{27}FN_4O_3S$; 421.1704, found 421.1628; HPLC Purity: 99.72 %.

4.2.28 Synthesis of (S)-1-((3-(3-fluoro-4-(2-oxa-6-azaspiro[3.3]heptan-6-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)-3-(4-nitrophenyl)thiourea (34): To a solution of **17** (100 mg, 0.325 mmol) in 5 mL DCM, TEA (181 µL, 1.302 mmol) was added and reaction mass was

cooled to 0 °C, 1-isothiocyanato-4-nitrobenzene (76 mg, 0.423 mmol) was added and stirred for 2 hrs at RT. Work up and purification was done similar to (28) to get pure product (87 mg). Yield: 54.8 %; yellow solid; m.p. 200-202 °C; ^{1}H-NMR (400 MHz, DMSO-*d6*) δ (ppm): 10.53 (s, 1H), 8.53 (s, 1H), 8.18 (d, *J* = 1.88Hz, 2H), (d , *J* = 2.92Hz, 2H), 7.42 (dd, *J* = 2.4Hz, 10.98 Hz, 1H), 7.14 (dd, *J* = 2Hz, 8.68Hz , 1H), 6.60 (dd, *J* = 8.9Hz, 10Hz, 1H), 4.94-4.90 (m, 1H), 4.70 (s, 4H), 4.13 (t, *J* = 9.0Hz, 1H), 4.01 (s, 4H), 3.93-3.89 (m, 2H), 3.82-3.80 (m, 1H), HRMS (ESI^{+}) *m/z* $[M+Na]^{+}$ calcd for $C_{22}H_{22}FN_5O_5S$ Na ; 510.1218, found 510.1271; HPLC Purity: 99.60%.

4.2.29 Synthesis of (1S,4R)-N-(((S)-3-(3-fluoro-4-(2-oxa-6-azaspiro[3.3]heptan-6-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)-4,7,7-trimethyl-3-oxo-2-oxabicyclo[2.2.1]heptane-1-carboxamide (35): To a solution of **17** (100 mg, 0.325 mmol) in 5 mL of DCM, TEA (181 µL, 1.302 mmol) added and reaction was cooled to 0 °C, (1S,4R)-4,7,7-trimethyl-3-oxo-2-oxabicyclo[2.2.1]heptane-1-carbonyl chloride (106 mg, 0.488 mmol) was added and stirred for 2 hrs at RT. Work up was done similar to compound (18) and purified by flash chromatography in DCM and MeOH (95:5) to get pure product (50 mg). Yield: 31.5 %; white solid; m.p. 103-105 °C; ^{1}H-NMR (300 MHz, DMSO-*d6*) δ (ppm): 8.39 (t, *J* = 3.6Hz, 1H), 7.39 (d, *J* = 14Hz, 1H), 7.08 (d, *J* =5.1Hz, 1H), 6.57 (d, *J* = 6Hz, 1H), 4.7 (s, 4H), 4.02 (s, 4H), 3.78 - 3.76 (m, 1H), 3.53-3.50 (m, 2H), 2.42-2.34 (m, 2H), 1.93-1.91 (m, 3H), 1.53-1.50 (m, 1H), 0.99 (s, 3H), 0.94 (s, 3H), 0.74 (s, 3H); LC-MS (ESI +ve) *m/z* 488.2 $[M+H]^{+}$; HRMS (ESI +ve) *m/z* $[M+H]^{+}$ calcd for: $C_{25}H_{30}FN_3O_6$; 488.2191, found 488.2204; HPLC Purity: 98.44 %.

4.2.30 Synthesis of (S)-N-((3-(3-fluoro-4-(2-oxa-6-azaspiro[3.3]heptan-6-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)adamantane-1-carboxamide (36): To a solution of **17** (100 mg, 0.325 mmol) in 5 mL of DCM, TEA (181 µL, 1.302 mmol) added and reaction was cooled to 0 °C, adamantane-1-carbonyl chloride (97 mg, 0.488 mmol was added and stirred for 2 hrs at RT. Work up was done similar to compound (18) and purified by flash chromatography in DCM and MeOH (95:5) to get pure product (90 mg). Yield: 58.9 %; white solid; m.p. 178-180 °C; ^{1}H-NMR (300 MHz, DMSO-*d6*) δ (ppm): 7.72 (t, *J* = 3.6Hz, 1H), 7.38 (d, *J* = 14Hz, 1H), 7.09 (d, *J* = 8.7Hz, 1H), 6.57 (d, *J* = 9.3Hz, 1H), 4.70 (s, 4H), 4.02 (s, 4H), 3.71-3.68 (m, 1H), 3.40-3.34 (m, 2H), 1.92 (s, 3H), 1.71-1.61 (m, 14H); ^{13}C-NMR (100 MHz, DMSO-*d6*) δ: 177.77, 154.17, 153.20, 135.44, 135.33, 130.19, 114.71, 106.87, 79.70(2C), 71.21, 62.28(2C), 47.48, 41.57,

40.14, 36.06(3C), 30.66(3C), 27.58(4C); LC-MS (ESI +ve) *m/z* 470.3 $[M+H]^+$; HRMS (ESI +ve) *m/z [*$M+H]^+$ calcd for: $C_{26}H_{32}FN_3O_4$; 470.2450, found 470.2490; HPLC Purity: 99.14 %.

4.2.31 Synthesis of (S)-N-((3-(3-fluoro-4-(2-oxa-6-azaspiro[3.3]heptan-6-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)-2-methoxyacetamide (37): To a solution of **17** (100 mg, 0.325 mmol) in 5 mL of DCM, TEA (181 µL, 1.302 mmol) was added and reaction mass was cooled to 0 °C, 2-methoxyacetyl chloride (44.56 µL, 0.488 mmol) was added and stirred for 2 hrs at RT. Work up was done similar to compound (18) and purified by flash chromatography in DCM and MeOH (95:5) to get pure product (70 mg). Yield: 52.7 %; white solid; m.p. 140-142 °C; ^{1}H-NMR (400 MHz, DMSO-*d6*) δ (ppm): 8.14 (t, *J* = 6Hz, 1H), 7.38 (dd, *J* = 2.36Hz, 10.98Hz, 1H), 7.11 (dd, *J* = 1.6Hz, 8.6Hz, 1H), 6.59 (t, *J* = 8.9Hz, 1H), 4.72 (m, 5H), 4.06-6.55 (m, 5H), 3.82 (s, 2H), 3.75-3.41 (m, 1H), 3.45 (m, 2H), 3.27 (s, 3H); HRMS (ESI +ve) *m/z* [M+H]+ calcd for $C_{18}H_{22}FN_3O_5$; 380.1616, found 380.1685; HPLC Purity: 97.87 %.

4.2.32 Synthesis of (R)-N-((3-(3-fluoro-4-(2-oxa-6-azaspiro[3.3]heptan-6-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)-1,2-dimethyl-1H-imidazole-4-sulfonamide (38): To a solution of **17** (100 mg, 0.325 mmol) in 5 mL of DCM, TEA (181 µL, 1.302 mmol) added and reaction was cooled to 0 °C, 1,2-dimethyl-1H-imidazole-4-sulfonyl chloride (101 mg, 0.521 mmol) was added and stirred for 2 hrs RT. Work up and purification was done similar to (23) to get pure product (60 mg). Yield: 39.6 %; white solid; m.p. 182-184 °C; ^{1}H-NMR (300 MHz, DMSO-*d6*) δ (ppm): 7.81 (t, *J* = 3.6Hz, 1H), 7.64 (s, 1H), 7.33 (d, *J* = 8.4Hz, 1H), 7.09 (d, *J* = 8.7Hz, 1H), 6.57 (d, *J* = 9.3Hz, 1H), 4.70 (s, 5H), 4.02 (s, 5H), 3.77-3.75 (m, 1H), 3.57 (s, 3H), 3.14-3.12 (m, 2H), 2.2 (s, 3H); ^{13}C-NMR (100 MHz, DMSO- *d6*) δ: 153.99, 152.41, 146.57, 136.89, 135.51, 135.40, 124.56(2C), 114.74, 106.91, 79.71(2C), 71.07, 62.27(2C), 47.11, 45.16, 40.12, 32.82, 12.43; LC-MS (ESI +ve) *m/z* 466.1 $[M+H]^+$; HRMS (ESI +ve) *m/z* $[M+H]^+$ calcd for: $C_{20}H_{24}FN_5O_5S$; 466.1555, found 466.1549; HPLC Purity: 99.14 %.

4.2.33 Synthesis of (R)-3,5-dichloro-N-((3-(3-fluoro-4-(2-oxa-6-azaspiro[3.3]heptan-6-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)benzenesulfonamide (39): To a solution of **17** (100 mg, 0.325 mmol) in 5 mL of DCM, TEA (181 µL, 1.302 mmol) added and reaction was cooled to 0 °C, 3,5-dichlorobenzene-1-sulfonyl chloride (120 mg, 0.488 mmol) was added and stirred for 2 hrs at RT. Work up and purification was done similar to (23) to get pure product (80 mg). Yield: 47 %; White solid; m.p. 105-107 °C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 8.40 (s, 1H),

7.97 (t, *J* = 2.1Hz, 1H), 7.80 (d, *J* = 1.8Hz, 2H), 7.38-7.33 (m, 1H), 7.07-7.05 (m, 1H), 6.61 (t, *J* = 10.2Hz, 1H), 4.70 (s, 5H), 4.02 (s, 5H), 3.70-3.68 (m, 1H), 3.19-3.18 (m, 2H); ^{13}C-NMR (50 MHz,$CDCl_3$) δ 154.52, 149.54, 142.76, 136.11(2C), 132.77(2C), 129.16, 125.30 (3C), 114.89, 107.66, 81.04(2C), 71.18, 62.78(2C), 47.48, 45.41, 39.81; MS: *m/z* 516.1 $[M+H]^+$; HRMS (ESI +ve) *m/z* $[M+H]^+$ calcd for: $C_{21}H_{20}C_{l2}FN_3O_5S$; 516.0558, found 516.0518; HPLC Purity: 99.29 %.

4.2.34 Synthesis of (R)-2-chloro-N-((3-(3-fluoro-4-(2-oxa-6-azaspiro[3.3]heptan-6-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)-5-nitrobenzenesulfonamide (40): To a solution of **17** (100 mg, 0.325 mmol) in 5 mL of DCM, TEA (181 µL, 1.302 mmol) added and reaction was cooled to 0 °C, 2-chloro-5-nitrobenzene-1-sulfonyl chloride (108 mg, 0.423 mmol) was added and stirred for 2 hrs at RT. Work up and purification was done similar to (23) to get pure product (76 mg). Yield: 44.3 %; brown solid; m.p. 98-100 °C; ^{1}H-NMR (400 MHz, DMSO-*d6*) δ (ppm): 8.81 (t, *J* = 8Hz, 1H), 8.62 (d, *J* = 2.72, 1H), 8.41 (dd, *J* = 2.72Hz, 8Hz, 1H), 7.89 (d, *J* = 8Hz, 1H), 7.29 (dd, *J* = 2.24Hz, 14.64 Hz, 1H), 7.0 (d, *J* = 10Hz, 1H), 6.57 (d, 8Hz, 1H), 4.70 (s, 4H), 4.63 - 4.58 (m, 1H), 4.02 (s, 4H), 4.0 (d, *J* = 8Hz, 1H), 3.65 - 3.63 (m, 1H), 3.87-3.32 (m, 2H); ^{13}C-NMR (100 MHz, DMSO-*d6*) δ: 153.58, 152.34, 145.98, 139.40, 137.21, 135.45, 133.39, 129.97, 128.16, 124.96, 114.66, 114.40, 106.72, 49.70(2C), 71.01, 62.24(2C), 46.88, 45.11, 40.12; HRMS (ESI +ve) *m/z* $[M-H]^-$ calcd for $C_{21}H_{20}ClFN_4O_7S$; 525.0642, found 525.0646; HPLC Purity: 98.49 %.

4.2.35 Synthesis of (S)-1-(4-chlorophenyl)-3-((3-(3-fluoro-4-(2-oxa-6-azaspiro[3.3]heptan-6-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)urea (41): To a solution of **17** (100 mg, 0.325 mmol) in 5 mL of DCM, TEA (181 µL, 1.302 mmol) added and reaction was cooled to 0 °C, 1-chloro-4-isocyanatobenzene (75.0 mg, 0.488 mmol) was added and stirred for 2 hrs at RT. Work up and purification was done similar to (25) to get pure product (80 mg). Yield: 53.3 %; off-white solid; m.p. 218-220 °C; ^{1}H-NMR (400 MHz, DMSO-d6) δ (ppm): 8.72 (s, 1H), 8.74-7.35 (m, 5H), 7.11 (dd, *J* = 1.9Hz, 8.7Hz, 1H), 6.58-6.50 (m, 2H), 4.75-4.73 (m, 1H), 4.69 (s, 4H), 4.08 (t, *J* = 18 Hz, 1H), 4.01-4.00 (d, *J* = 1.6Hz , 4H), 3.72-3.68 (m, 1H), 3.46 (t, *J* = 5.5Hz, 2H); HRMS (ESI +ve) *m/z* [M+H]+ calcd for $C_{22}H_{22}ClFN_4O_4$; 461.1431, found 461.1404; HPLC Purity: 98.31 %.

4.2.36 Synthesis of (S)-1-(4-chlorophenyl)-3-((3-(3-fluoro-4-(2-oxa-6-azaspiro[3.3]heptan-6-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)thiourea (42): To a solution of **17** (100 mg, 0.325 mmol) in 5 mL of DCM, TEA (181 µL, 1.302 mmol) added and reaction was cooled to 0 °C, 1-chloro-4-isothiocyanatobenzene (83 mg, 0.488 mmol) was added and stirred for 2 hrs at RT. Work up and purification was done similar to (28) to get pure product (80 mg). Yield: 51.5 %; white solid; m.p. 200-202 °C; ^{1}H-NMR (300 MHz, DMSO-d6) δ (ppm): 9.78 (s, 1H), 8.11 (s, 1H), 7.46 (d, *J* = 8.7Hz, 1H), 7.36 (d, *J* = 8.7Hz, 2H), 7.14 (d, *J* = 8.7Hz, 2H), 6.62 (t, *J* = 9.3Hz, 1H), 6.62-6.58 (m, 1H), 4.91-4.90 (m, 1H), 4.70 (s, 4H), 4.09-4.06 (m, 1H), 4.0 (s, 4H), 3.88-3.82 (m, 2H), 3.18 (d, *J* = 5.1Hz, 1H); ^{13}C-NMR (50 MHz, DMSO-d6) δ: 181.36, 154.06, 153.61, 138.08, 135.63, 135.41, 130.16(4C), 124.85, 114.67(2C), 107.14, 79.72(2C), 71.05, 62.29(2C), 47.39, 46.45, 42.10; HRMS (ESI +ve) *m/z* $[M+H]^+$ calcd for $C_{22}H_{22}ClFN_4O_3S$; 477.1158, found 477.1172; HPLC Purity: 98.66 %.

4.2.37 Synthesis of (1R,5S)-tert-butyl 3-(4-((S)-5-(acetamidomethyl)-2-oxooxazolidin-3-yl)-2-fluorophenyl)-8-azabicyclo[3.2.1]oct-2-ene-8-carboxylate (45): To a solution of **43** (100 mg, 0.302 mmol) in 5mL of dioxane and 1 mL of water, added ((1R,5S)-8-(tert-butoxycarbonyl)-8-azabicyclo[3.2.1]oct-2-en-3-yl)boronic acid(42) (99 mg, 0.393 mmol), Added sodium carbonate (112 mg, 1.057 mmol), Argon gas was purged for 20 minutes. Then added PdCl2 (dppf)-CH2Cl2 adduct (12.33 mg, 0.015 mmol). Again argon gas was purged into reaction mass. Heated the reaction mass for 100 °C for overnight. Then concentrated the reaction mass. Product was dissolved into DCM. Saturated sodium bicarbonate solution washing was given to organic layer followed by water washing was given. The compound was purified by flash chromatography in DCM and MeOH (95:5) to get pure product (60 mg). Yield: 43.2 %; off-white solid; m.p. 155-158 °C; ^{1}H-NMR (300 MHz, DMSO-*d6*) δ (ppm): 8.25 (t, *J* = 5.56Hz, 1H), 7.48 (d, *J* = 14Hz, 1H), 7.33-7.27 (m, 2H), 6.32 (s, 1H), 4.71-4.70 (m , 1H), 4.35-4.30 (m, 2H), 4.10 (t, *J* = 9Hz, 1H), 3.74-3.72 (m, 1H), 3.40 (t, *J* = 5.4Hz, 2H) 2.92-2.90 (m, 1H), 2.19-2.13 (m , 2H), 1.92-1.91 (m, 2H), 1.82 (s, 3H), 1.72-1.70 (m, 1H), 1.39 (s, 9H); ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 171.23, 162.57, 157.64, 154.17, 138.04, 132.54, 129.67, 124.29, 113.15(2C), 106.43, 79.43, 72.00(2C), 47.38(2C), 41.84(2C), 37.28, 34.29, 28.36(3C), 22.99; LC-MS (ESI +ve) *m/z* 460.2$[M+H]^+$; HRMS (ESI +ve) *m/z* $[M+H]^+$ calcd for $C_{24}H_{30}FN_3O_5$; 460.2242, found 460.2201; HPLC Purity: 99.32 %.

4.2.38 Synthesis of 5-benzyl-1-oxa-5-azaspiro[2.4]heptanes (47): To a solution of trimethylsulfoxonium iodide (7.4 g) was added to a suspension of NaH (0.81 g) in anhydrous DMSO (25 mL) at 10 to 15 °C over twenty minutes, followed by stirring at room temperature under nitrogen atmosphere for 1 hr. A solution of **46** (4.9 g) (commercial available) in anhydrous DMSO (25 mL) was added thereto at 10 to 15 °C over twenty minutes, followed by stirring at 10 to 24 °C for one hr. Water (50 mL) and EtOAc (50 mL) were added thereto under cooling with ice-water, and the mixture was separated. The product was extracted with EtOAc (50 mL x 2) and was combined with the former organic layer. The combined organic layer was sequentially washed with water (30 mL x 3) and brine (50 mL x 1) and was dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure, the residue was purified by silica gel column chromatography (eluent; hexane: EtOAc = 1:1) to yield the title compound (2.25g) as an oil. Yield: 44.4%; brown semi solid; ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 7.34-7.32 (m, 5H), 3.72 (q, *J* = 12.3Hz, 2H), 2.82-2.80 (m, 2H), 2.63-2.56 (m, 2H), 2.26-2.17 (m, 2H), 1.95-1.88 (m, 2H); LC- MS(ESI +ve) *m/z* 190 $[M+H]^+$; HPLC Purity: 99.39%.

4.2.39 Synthesis of 1-benzyl-3-(((3-fluoro-4-morpholinophenyl)amino)methyl) pyrrolidin-3-ol (49): To a solution of **47** (601.5 mg, 3.18 mmol) in 10 mL of ACN, added the lithium perchlorate (676 mg, 6.36 mmol), added **48** (686 mg, 3.50 mmol) and heated the reaction mass for 90 °C overnight. Then concentrated the reaction mass and purification was done in DCM and MeOH to give the title compound (700 mg). Yield: 57.1%; brown semi solid; ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 7.32 (s, 5H), 6.82 (t, *J* = 9Hz, 1H), 6.48 (d, *J* = 2.1Hz,1H), 6.34 (d, *J* = 8.4Hz, 1H), 5.36 (br s, 1H), 4.9 (br s, 1H), 4.0 (m, 1H), 3.69-3.66 (t, *J* = 4.2 Hz , 5H), 3.16 (d, *J* = 5.1Hz, 1H), 3.01 (s, 2H), 2.81-2.72 (t, *J* = 4.5Hz, 6H), 1.98-1.47 (m , 3H); ^{13}C-NMR (100 MHz, DMSO-*d6*) δ: 157.60, 146.40, 138.94, 128.86, 128.47(3C), 126.76(2C), 120.41, 107.60, 100.19, 79.16, 66.38(3C), 64.73, 59.63, 52.87, 52.78(2C), 30.66; LC-MS (ESI +ve) *m/z* 386.2 $[M+H]^+$; HRMS (ESI +ve) *m/z* $[M+H]^+$ calcd for $C_{22}H_{28}FN_3O_2$: 386.2238, found 386.2242; HPLC Purity: 98.68%

4.2.40 Synthesis of 7-benzyl-3-(3-fluoro-4-morpholinophenyl)-1-oxa-3,7-diazaspiro[4.4] nonan-2-one (50): To a solution of **49** (500 mg, 1.297 mmol), added di(1H-imidazol-1-yl)methanone (421 mg, 2.59 mmol), added 2,3,4,6,7,8,9,10-octahydropyrimido[1,2-a]azepine (494 mg, 3.24 mmol). After stirring at 80° C. overnight, the solvent was evaporated under reduced pressure. The residue was dissolved in EtOAc, and the solution was washed twice with

aqueous sodium hydrogen carbonate solution. The organic layer was dried over sodium sulphate, and the solvent was evaporated under reduced pressure. The residue was purified using flash chromatography using EtOAc and pet ether to give the title compound (400 mg). Yield: 47.9%; off-white solid, m.p. 148-150 °C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 7.4 (s. 1H), 7.43 (s, 1H), 7.32 (s, 3H), 7.25 (m, 1H), 7.19-7.16 (d, *J* = 9Hz, 1H), 7.07-7.01 (t, *J* = 9.6Hz, 1H), 4.0 (s, 2H), 3.7 (s, 3H), 3.6 (s, 2H), 2.9 (s, 4H), 2.85 (s, 1H), 2.74-2.6 (m, 3H), 2.54 (s, 1H), 2.16 (m, 1H), 1.26 (d, *J* = 10.8Hz, 1H), ^{13}C-NMR (100 MHz, DMDO-*d6*) δ: 155.74, 153.55, 138.49, 133.47, 133.37, 128.49(3C), 126.93(2C), 119.19, 114.25, 106.95, 83.98, 66.13(2C), 64.25, 58.98, 54.58, 50.70, 50.67, 37.79, 30.66; LC-MS (ESI +ve) *m/z* 412.7 $[M+H]^+$; HRMS (ESI +ve) *m/z* $[M+H]^+$ calcd for $C_{23}H_{26}FN_3O_3$: 412.2038, found 412.2031; HPLC Purity: 98.59%.

4.2.41 Synthesis of 3-(3-fluoro-4-morpholinophenyl)-1-oxa-3,7-diazaspiro[4.4]nonan-2-one (51): To a solution of **50** (1.5 g, 3.65 mmol), In MeOH, added palladium hydroxide on carbon (0.358 g, 2.55 mmol). Stirred the reaction mass overnight at rt at hydrogen atmosphere by using hydrogen balloon. Then filtered the reaction mass and concentrated the reaction mixture. For further purification was done in DCM and MeOH by using flash chromatography to yield the title compound (600 mg) Yield: 51.2%; brown solid, m.p. 120-122 °C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 7.51 (d, d, *J* = 1.2Hz, 1.2Hz, 1H), 7.21 (d, d, *J* = 1.2Hz, 1.2Hz, 1H), 7.09 (t, *J* = 5.7Hz, 1H), 4.10 (q, *J* = 5.4Hz, 12.3Hz, 2H), 3.74 (m , 4H), 3.14 (d, *J* = 2Hz, 1H), 3.0-2.93 (m, 7H), 2.17 (m, 1H), 1.99 (m, 1H), 1.90 (s, 1H); ^{13}C-NMR (300 MHz, DMSO-*d6*) δ: 155.78, 153.66, 135.15, 121.62, 120.27, 119.15, 106.62, 85.95, 66.12(2C), 57.69, 52.53, 50.66(2C), 45.41, 30.60; LC-MS (ESI +ve) *m/z* 322.3 $[M+H]^+$; HRMS (ESI +ve) *m/z* $[M+H]^+$ calcd for $C_{16}H_{20}FN_3O_3$: 322.1555, found 322.1561; HPLC Purity: 98.70%.

4.2.42 Synthesis of 7-acetyl-3-(3-fluoro-4-morpholinophenyl)-1-oxa-3,7-diazaspiro [4.4]nonan-2-one (52): To a solution of **51** (40 mg, 0.124 mmol) in 5 mL of THF, added the TEA (52.0 µL, 0.373 mmol), acetic anhydride (23.53 µL, 0.249 mmol) at 0° C, then stirred the reaction mass for rt for 1h. Then concentrated the reaction mass and water washing was given, extracted the product with EtOAc and purification was done with DCM and MeOH using flash chromatography to yield the title compound (30 mg) Yield: 66.3%, off-white solid; m.p. 83-85 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.51(d, *J* =14.8Hz, 1H, Ar-H), 7.20(m, 1H),7.09-7.052(t, *J* = 9.6, 1H, Ar-H),4.15-4.05(m, 2H), 3.90(d, *J* = 12H, 1H), 3.79-3.73(m, 5H), 3.57(m, 1H), 3.45(d, *J* = 13.2Hz, 1H), 2.96(s, 4H), 2.37-2.12(m, 2H), 1.97(d, *J* = 13.2Hz, 3H), LC-MS

(ESI +ve) *m/z* 364.3 $[M+H]^+$; HRMS (ESI +ve) *m/z* $[M+H]^+$ calcd for $C_{18}H_{22}FN_3O_4$: 364.1720, found 364.1667; HPLC Purity: 99.78%;

4.3 Biological evaluation

4.3.1 General

For biological activity, stock solutions of the compounds were prepared in DMSO (10 mg/mL) and were further used for testing antibacterial activity. Bacterial strains *E. coli* (NCIM 2688), *P. aeruginosa* (NCIM 2036) as Gram-negative and *B. subtilus* (NCIM 2079), *S. aureus* (NCIM 2010) as Gram-positive were obtained from NCIM (NCL, Pune) and were grown in Luria Burtony medi-um from Himedia, India. Antibacterial activity. Bacterial strains were grown to logarithmic phase (O.D. 1.0 at 620 nm) in a LB medium. Test compound solutions were added into 96-well plate. DMSO (1%) was used as vehicle control. Initially the compound concentrations of 30, 10 and 3 µg/mL were used to check the antibacterial activity. The compounds showing inhibition above 75% for 3 µg/mL concentration were selected for further analysis by dose-response curve. Dose-response curves were generated by using half dilution from 100 µg/mL upto 0.78 µg/mL. The OD was measured by Spectramax spectrophotometer at 620 nm.

4.3.2 Antibacterial activity

All bacterial cultures were first grown in LB media at 37 °C at 180 RPM. The assay was performed in 96-well plates after 8 and 12 hrs. for Gram-negative and Gram-positive bacteria, respectively. A 0.1 % suspension of OD 1 (620 nm) culture was used for screening. The inoculated culture was added into each well of 96-well plate containing the compounds to be tested. Optical density for each plate was measured at 620 nm after 8 hrs for Gram-negative bacteria and after 12 hrs for Gram-positive bacteria. For antibacterial assay, 2.5 µL of test compound solution was added in each of the well of 96-well plates, except control and blank followed by 247.5 µL of inoculated culture to each well except blank to make the final volume of 250 µL. DMSO (2.5 µL) was used as vehicle control in the blank and control. Sterile media (248 µL) was added into blank well.

4.3.3 Antimycobacterial activity

Microbial strains such as *Mycobacterium tuberculosis* H37Ra (ATCC 25177) and *M. bovis* BCG (ATCC 35734) were obtained from AstraZeneca, India. The stock culture was maintained at -80 ^{o}C and subcultured once in a liquid medium before inoculation into an experimental culture. Cultures were grown in Dubos media (enrichment media). For antimycobacterial assay, *M. pheli* medium (minimal essential medium) was used. It contains 0.5 g KH_2PO_4, 0.25 g trisodium citrate, 60 mg MgSO4, 0.5 g aspargine and 2 mL glycerol in distilled water (100 mL) followed by pH adjustment to 6.6.

Cultivation of mycobacteria and antimycobacterial assay. All bacterial stock cultures were first grown in Dubos media at 37 ^{o}C at 150 RPM. It takes at least 8-10 days for OD 1 at 620 nm. The antimycobacterial assay was performed in 96-well plates for active as well as dormant stages.For antimycobacterial assay, 1 % of OD 1 culture at 620 nm was used. A total of 2.5 µL of test compound solution was added into 96-well plates, except control and blank, followed by 250 µL of inoculated culture to each well except blank. DMSO was used as vehicle control in blank and control. A total of 250 µL sterile media was added into the blank well. Sterile sealer was kept on it to achieve dormant condition. After 8 days, all oxygen was used by bacteria and hypoxic conditions were obtained, resulting in mycobacteria shifting to the dormant stage. XTT (for *Mtb*) as well as NR (for *M. bovis* BCG) assays were performed on 8th day for active stage inhibitor and on 12th day for dormant stage inhibitor. Primary screening was done at three concentrations 30, 10, 3 µg/mL. Those compounds showing > 80% inhibition at 3 µg/mL concentration were selected further for generating dose-response curves using half dilution from 100 µg/mL up to 0.78 µg/mL.

4.3.4 XTT assay

For *Mtb*, XTT assay was performed [22]. For active stage inhibitor, the plate was removed after 8 days. XTT sodium salt powder was used for preparing 1.25 mM stock solution in sterile 1X PBS and Menadione (Sigma) was prepared as a 6 mM solution in DMSO and was used immediately. XTT solution was added to make the final concentration to 200 µM. Plates were kept in 37 ^{o}C incubator for 20 min. Then menadione solution was added to make final concentration of 60 µM. The plates were incubated for 40 min in the incubator. The OD was measured at 470 nm. For dormant stage inhibitor, plates were removed after 12 days. All other procedures were same.

4.3.5 Nitrate Reductase assay

For *M. bovis* BCG, NR assay was performed using 1% sulfalic acid in 20% HCl and 1% NEED (1-naphthyl ethylenediamine dihydrochloride) in water. After 8^{th} and 12^{th} days, the plates were removed for active and dormant stage inhibitors, respectively. A solution containing one part each of culture from assay plate, sulfanilic acid solution and NEED solution was prepared. Purple colour prontosil produced was measured at 540 nm.

Percent inhibition was measured as -

$$\% \text{ Inhibition} = \frac{\text{Comp. OD} - \text{Blank}}{\text{Cont OD} - \text{Blank}}$$

Acknowledgement

PG thanks Niteshkumar Sahu, Chetan Shah and Ms. Sona Warrier, PhD Students from PSK's Research Group at SPPSTM, SVKM's NMIMS, Mumbai, for their help during preparation of this manuscript. Authors are also thankful to Schrödinger Inc. for providing the Demo license of Schrödinger Molecular Modeling Suite and especially Craig Coel for his valuable support that has tremendously helped in this work. Authors also thankful to SIF VIT University for providing NMR facility.

Supplementary material

Experimental details of synthesis and characterization of representative compounds using ^{1}H-NMR, ^{13}C-NMR, Mass spectrometry, HPLC Purity and HRMS are provided.

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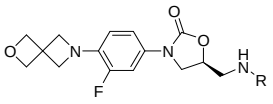
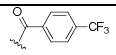
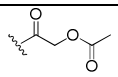
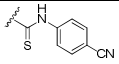
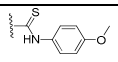
FIGURE CAPTIONS

Figure 1. Structures of approved and developmental oxazolidinone antibacterials

Figure 2. 2-Oxa-6-azaspiro[3.3]heptanes, a bioisostere of morpholine

Table 1: Antibacterial activity data of the title compounds

Compound No.	 R	*E. coli*		*Pseudomonas aeruginosa*		*Staphylococcus aureus*		*Bacillus subtillus*	
		MIC µg/mL	IC_{50} µg/mL	MIC µg/mL	IC_{50} µg/mL	MIC µg/mL	IC_{50} µg/mL	MIC µg/mL	IC_{50} µg/mL
1	-	1.15	0.49	1.31	0.49	1.13	0.42	1.8	0.64
17	H	4.05	0.57	>100	>100.	3.38	0.843	>100	>100
18		>100	>100	6.4	0.58	2.90	0.53	3.46	0.65
19		8.33	0.67	>100	>100	18.26	1.03	>100	>100
20		>100	>100	18.14	1.19	22.31	0.67	>100	>100
21		>100	>100	>100	>100	8.40	0.75	>100	>100
22		4.60	0.72	2.57	0.51	2.05	0.88	2.35	0.49
23		15.42	0.89	3.52	1.13	1.31	0.46	2.20	0.64
24		3.03	0.57	>100	>100	>100	>100	>100	>100
25		>100	>100	19.86	0.814	>100	>100	14.78	0.76
26		10.45	0.64	23.96	1.25	16.21	0.88	13.07	0.65
27		1.36	0.49	>100	>100	>100	>100	15.02	1.53
28		9.54	1.00	>100	>100	11.23	1.03	3.87	0.86
29		2.41	0.49	>100	>100	>100	>100	>100	>100
30		>100	>100	32.28	1.27	>100	>100	6.04	0.64
31		10.67	1.10	>100	>100	16.13	0.74	8.78	1.10

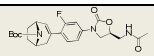
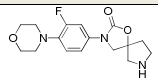
32		>100	>100.	15.90	0.98	5.76	0.60	5.18	1.31
33		22.78	1.38	>100	>100	9.15	1.45	>100	>100
45		3.66	0.57	15.09	0.67	2.95	0.74	5.67	0.55
51		0.554	4.437	>100	>100	>100	>100	>100	>100

Table 2: Antitubercular activity data of the title compounds

Compound No.	*Mycobacterium tuberculosis H37Ra*				*Mycobacterium bovis BCG*			
	Active		**Dormant**		**Active**		**Dormant**	
	MIC (µg/mL)	**IC_{50} (µg/mL)**	**MIC (µg/mL)**	**IC_{50} (µg/mL)**	**MIC (µg/mL)**	**IC_{50} (µg/mL)**	**MIC (µg/mL)**	**IC_{50} (µg/mL)**
1	1.31	0.8	1.25	0.71	0.42	0.029	0.43	0.31
17	18.27	2.8	11.33	3.46	29.49	15.72	24.35	17.34
18	2.55	1.56	3.10	2.07	1.83	0.79	1.67	0.81
21	7.80	3.24	10.63	6.69	6.32	3.35	5.77	3.05
22	10.89	6.4	21.23	9.58	9.82	4.11	10.12	4.83
32	41.12	16.34	41.63	22.83	44.46	13.07	61.18	17.67
45	21.4	12.33	20.49	10.84	31.16	4.35	23.14	5.82

Table 3: *In silico* ADME prediction data of the title compounds

Molecule	MW[a]	%Human oral Absorption[b]	Hbond Donor[c]	Hbond acceptor[d]	logPo/w[e]	logS[f]	logBB[g]	logKhsa[h]
1	337.35	77.18	1	8.7	0.49	-2.10	-0.58	-0.62
17	307.32	78.42	2	6.5	1.36	-2.27	0.04	-0.11
18	349.36	82.47	1	8	1.50	-3.28	-0.63	-0.41
19	412.42	90.14	1	9.5	2.49	-4.79	-0.90	-0.05
20	479.43	100	1	8	4.42	-6.99	-0.39	0.53
21	407.39	74.24	1	10	1.34	-4.07	-1.39	-0.49
22	402.38	81.33	1	9.5	1.88	-4.42	-1.20	-0.19
23	487.94	86.76	1	10	2.64	-4.87	-1.00	-0.04
24	515.47	77.77	1	10	3.18	-5.38	-0.87	0.12
25	444.43	94.44	2	7.5	3.37	-6.13	-0.76	0.22
26	494.44	100	2	7.5	4.05	-7.07	-0.61	0.40
27	444.43	94.89	2	7.5	3.28	-5.73	-0.69	0.19
28	467.51	89.44	2	9.5	3.31	-7.58	-1.35	0.30
29	510.50	85.72	2	8	5.01	-7.96	-0.21	0.71
30	408.49	100	2	8	3.53	-6.20	-0.42	0.34
31	472.53	100	2	8.75	4.16	-6.84	-0.53	0.47
32	460.49	100	2	8	4.28	-6.74	-0.25	0.49
33	408.49	100	2	8	3.53	-6.20	-0.42	0.34
45	459.51	90.29	1	8.5	3.45	-6.09	-1.08	0.39
51	321.35	81.51	1	7.7	1.41	-2.69	0.25	-0.15

[a]Molecular weight(130.0/725.0), [b]% Human Oral Absorption in GI (<25% is poor). [c]# of H-bond donor groups (0/6), [d]# H-bond acceptor groups (2/20), [e]octanol/water partition coefficient (-2.0/6.5), [f]predicted aqueous solubility (-6.5/0.5), [g]predicted brain/blood partition coefficient (-3.0/1.2); [h]prediction of binding to human serum albumin (-1.5/1.5); The values in bracket signify Range for 95% of drugs.

FIGURES

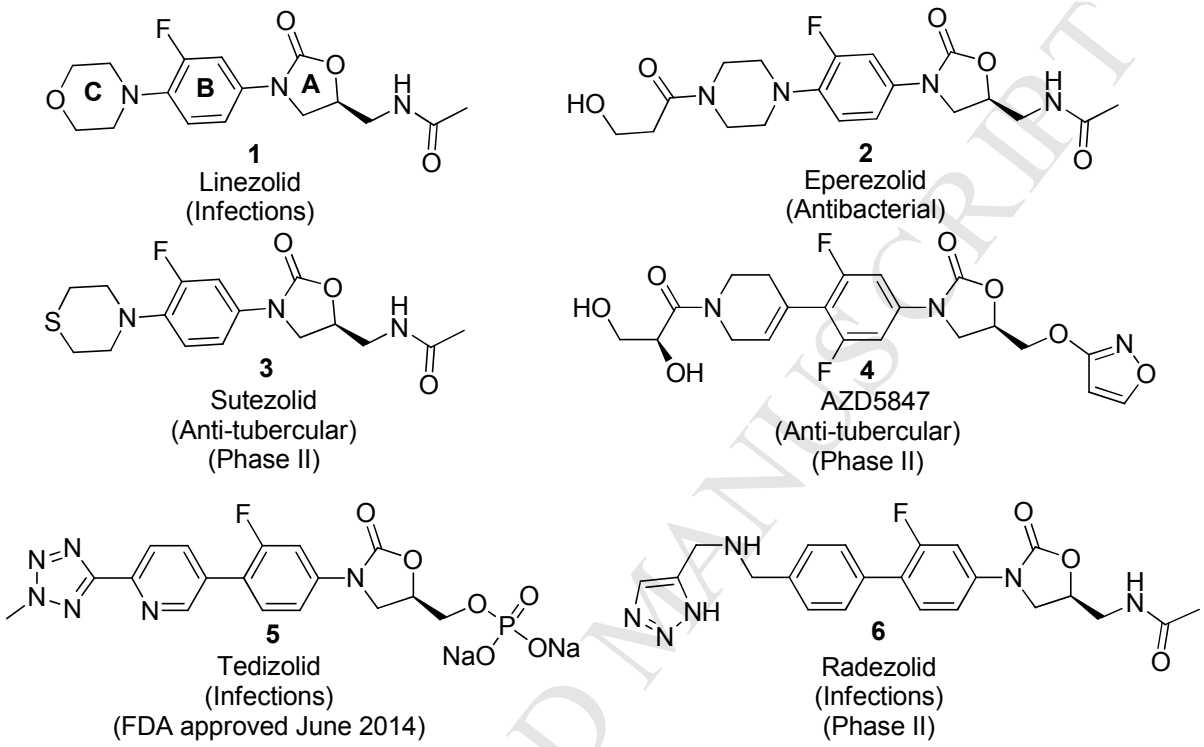


Figure 1. Structures of approved and developmental oxazolidinone anti-bacterial

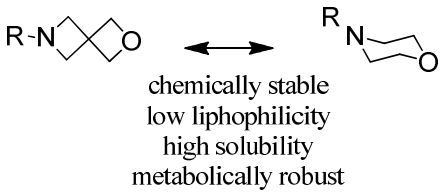
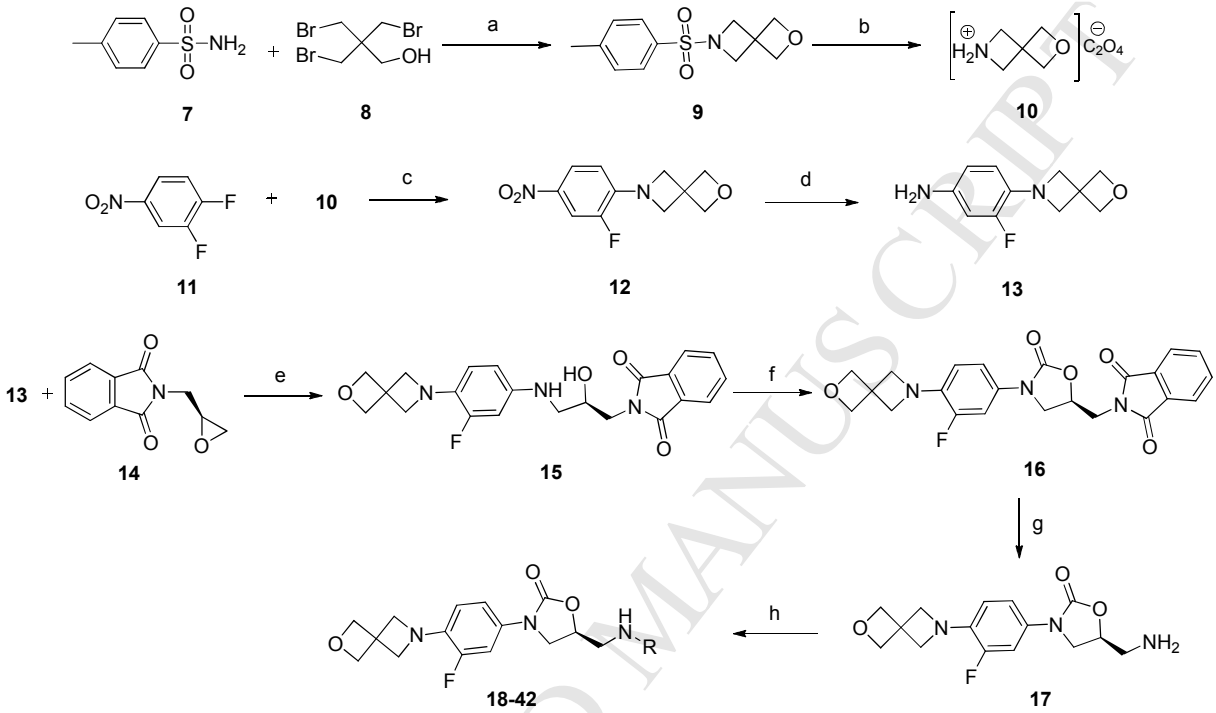


Figure 2. 2-Oxa-6-azaspiro[3.3]heptanes, a bioisostere of morpholine

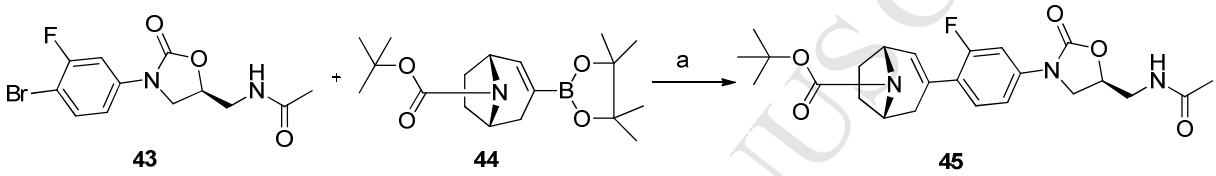
Schemes

Scheme 1.[a] Synthesis of 2-oxa-6-azaspiro[3.3]heptane analogue of **1**



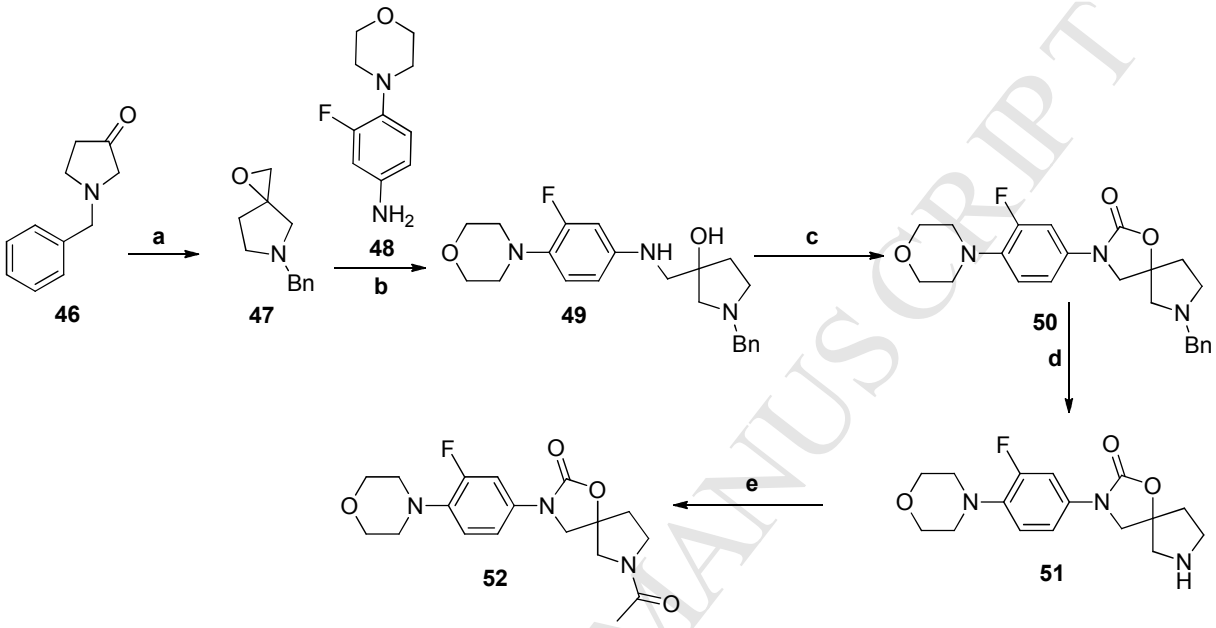
[a]Reagents and conditions (a) KOH, EtOH reflux at 90 °C for 48 hrs, 57.7%; (b) Mg turnings, MeOH, sonication for 1-2 hrs, 51.1%; (c) ACN, DIPEA, reflux at 90 °C overnight, 53.4%; (d) Pd-C, H_2 Parr® shaker, 4h RT at 65-80 PSI, 92%; (e) DIPEA, DMF, 120 °C overnight, 54.9%; (f) CDI, DMAP,THF, 60 ºC, 12 hrs, 61.1%; (g) Hydrazine hydrate, EtOH, reflux, 1h, 71.2%; (h) TEA, acetyl chloride, DCM, 0 °C, 1-2h, 79%

Scheme 2.[a] Synthesis of compound **45**.



[a] Reagents and conditions (a) Na_2CO_3, $PdCl_2$(dppf)-CH_2Cl_2adduct, 1,4-dioxane, water 100 °C, overnight, 43.2%.

Scheme 3. [a] Synthesis of compound **52.**



[a]Reagents and conditions (a) Trimethylsulfoxonium iodide, DMSO, NaH, 0 ^{o}C; 2h, 38%; (b) amine compound **48** , lithium perchlorate, ACN, 80 ^{o}C, reflux 12 hrs; 57 %; (c) CDI, DMAP,THF, 60 ^{o}C, 12 hrs, 48 % ; (d) $Pd(OH)_2$ H_2 atm, 12h, 51.2 % ; (e)TEA, 0 ^{o}C, 2h, Ac_2O, THF, 66.3%.

Highlights

- Synthesis of novel series of azaspiro analogues of linezolid
- Anti microbial activity studies of azaspiro analogues of linezolid
- Anti mycobaterial screening of azaspiro analogues of linezolid on *Mycobacterium tuberculosis* H37Ra (ATCC 25177) and *M. bovis* BCG (ATCC 35734)
- The *N*-acetyl derivative **18** was identified as lead molecule possessing good antitubercular profile.