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Preliminary communication

Synthesis of 1-[3-(4-benzotriazol-1/2-yl-3-fluoro-phenyl)-2-oxo-oxazolidin-5-ylmethyl]-3-substituted-thiourea derivatives as antituberculosis agents

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Abstract

In continuation of our research program for new antituberculosis drugs, we have designed, synthesized and evaluated antimycobacterial activity of new series of 1-[3-(4-benzotriazol-1/2-yl-3-fluoro-phenyl)-2-oxo-oxazolidin-5-ylmethyl]-3-substituted-thiourea derivatives against different Mycobacterium species i.e. *M. tuberculosis*, *M. avium* and *M. intracellulare* in an agar dilution method. Compound **17** exhibited excellent antimycobacterial activity (in vitro) against drug sensitive and resistant clinical isolates of *M. tuberculosis*. Its MIC value is equivalent to linezolid and superior to isoniazid against all these strains.

Keywords: Tuberculosis; Antituberculosis activity; Oxazolidinone derivatives; Benzotriazole substituent; Synthesis

1. Introduction

Tuberculosis (TB) is one of the leading causes of death due to a single infectious organism in the world. As per survey reported by Global Alliances, Geneva, there are 8–10 million new active cases of TB and approximately 3 million deaths each year [1–3]. The World Health Organization (WHO) has predicted that by year 2020 there will be 1 billion new active cases if new anti-TB drugs or treatments are not developed [4]. Treatment of TB infection that has been caused by multi drug resistant (MDR), *Mycobacterium (M.) tuberculosis* has become major concern world over. MDR TB and its synergy with HIV in immunocompromised patients have deteriorated the problem [5–13]. TB is therefore a leading cause of death among people who are HIV positive [14]. The increasing rate of MDR TB does not only create problems for treatment, but also costs are exploding. Since in the last 40 years there has not been a new drug for TB introduced in the market, there is an unmet need to discover new synthetic lead molecules and drugs that shorten the duration of therapy and to combat MDR TB.

The oxazolidinones, a new class of synthetic antimicrobial agents, are active against numerous MDR Gram-positive organisms [15]. Particularly, problematic pathogens include methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), penicillin- and cephalosporin-resistant *Streptococcus pneumoniae*. Their mode of action has been found to inhibit the protein synthesis in the initial stage [16]. Due to this novel mechanism of action; oxazolidinones are not cross resistant with other types of antibiotics [17]. Linezolid (Fig. 1), developed by Pharmacia & Upjohn, is the first compound of this class, commercialized world wide for the treatment of MDR Gram-positive infections. The thiomorpholine analogue of linezolid, PNU-100480 (Fig. 1) showed an interesting antimycobacterial activity [18,19]. We have earlier reported our research efforts towards the discovery of new chemical entities as potential antituberculosis agents [20–22]. In view of the above and in continuation of our ongoing program to develop anti-TB drugs, we have designed,

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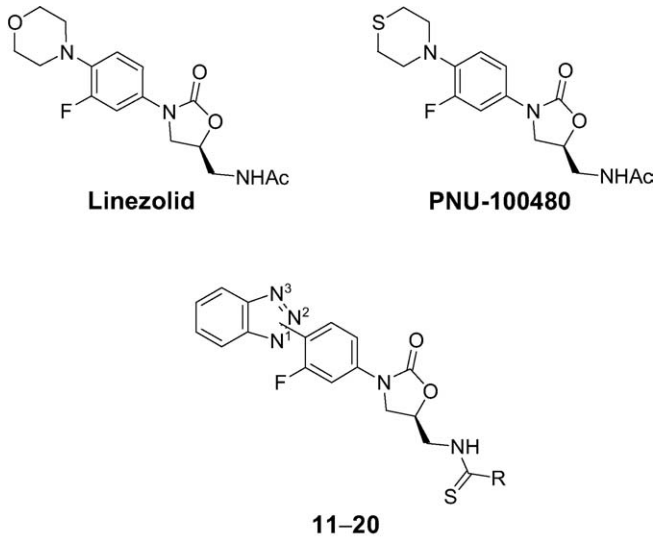


Fig. 1.

synthesized and evaluated antimycobacterial activity of new series of 1-[3-(4-benzotriazol-1/2-yl-3-fluoro-phenyl)-2-oxo-oxazolidin-5-ylmethyl]-3-substituted-thiourea derivatives **11**–**20** (Fig. 1), which we wish to report in this communication.

2. Chemistry

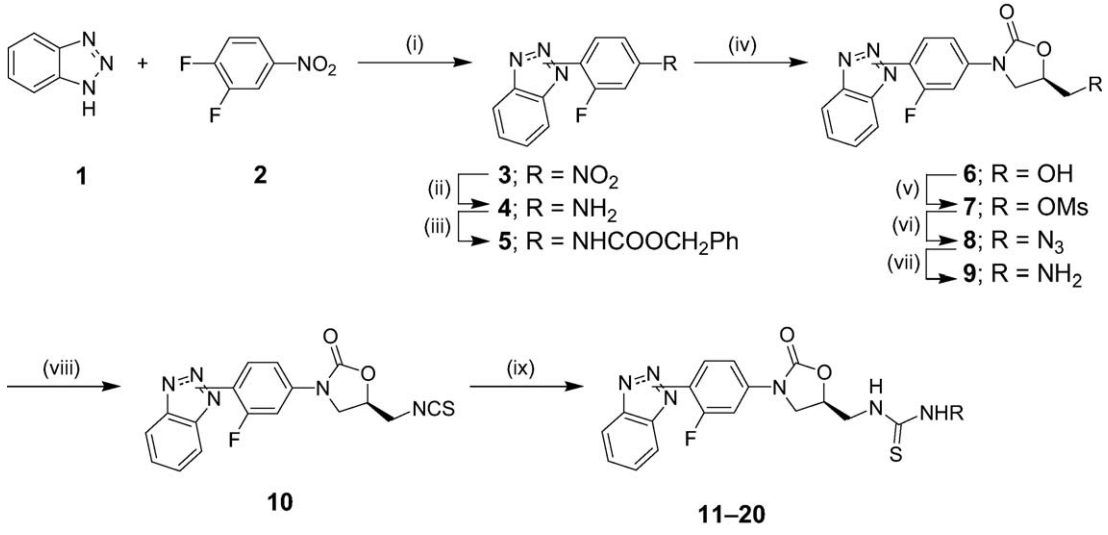
The synthesis of 1-[3-(4-benzotriazol-1/2-yl-3-fluoro-phenyl)-2-oxo-oxazolidin-5-ylmethyl]-3-substituted-thioureas **11**–**20** is outlined in Scheme 1.

Compounds **11**–**20** were synthesized starting from benzotriazole **1**. Reaction of **1** with 1,2-difluoro-4-nitrobenzene (**2**) was carried out under basic conditions to yield nitro compound **3**. The nitro group of compound **3** was reduced with Pd-C/H_2 to afford the amino compound **4**, followed by condensation with benzyl chloroformate to furnish (4-benzotriazol-1/2-yl-3-fluoro-phenyl)-carbamic acid benzyl ester **5**. Conversion of compound **5** to (5*R*)-3-(4-benzotriazol-1/2-yl-3-fluoro-phenyl)-5-hydroxymethyl-oxozolidin-2-one (**6**) was accomplished by use of *n*-butyl lithium and (*R*)-glycidyl butyrate in THF at –78 °C. Alcohol **6** was then converted to azide **8** via mesylate intermediate **7**. Azide **8** was reduced to amine **9** by using PPh_3, which on reaction with carbon disulfide solution and ethyl chloroformate gave the key intermediate (5*S*)-3-(4-benzotriazol-1/2-yl-3-fluoro-phenyl)-5-isothiocyanato-methyl-oxazolidin-2-one (**10**). This key intermediate **10** was used subsequently to make thiourea derivatives **11**–**20** by reacting it with appropriate primary amines (Scheme 1).

All new compounds reported here were fully characterized on the basis of complementary spectroscopic (^{1}H-NMR and MS) and analytical data. In ^{1}H-NMR, appearance of sets of peaks of the protons in the aromatic region indicated that arylation occurred at both N-1 and N-2 positions of benzotriazole (**1**). This type of pattern has been observed in ^{1}H-NMRs of all the intermediates (**3**–**10**) and final compounds (**11**–**20**) of this series. Such types of observation have been reported in literature also [23,24]. Separation of these positional isomers was very difficult by column chromatography or crystallization.

3. Microbiology

The antimycobacterial activity of the compounds was determined with the objective to identify the compounds having inhibitory activity against susceptible (sensitive strains; inhibited by the two front line anti-TB drugs viz. isoniazid, rifampicin) and resistant strains (not inhibited by either isoniazid or rifampicin or by both) of *M. tuberculosis* (causative agent of human tuberculosis). In addition to *M. tuberculosis*, the antimycobacterial activity was also evaluated against *M. avium* and *M. intracellulare* which are primary causative agents for avian tuberculosis but are also associated with the disease in humans



Scheme 1. Reagents and conditions: (i) K_2CO_3, DMF, 25–30 °C, 7 h; (ii) 10 % Pd–C, HCO_2NH_4, THF–MeOH, 25–30 °C, 4 h; (iii) Cbz-Cl, Na_2CO_3, Acetone–H_2O (2:1), 0→30 °C, 4 h; (iv) *n*-BuLi, (*R*)-glycidyl butyrate, THF, –78→30 °C, 12 h; (v) MsCl, Et_3N, DCM, 0→30 °C, 4 h; (vi) NaN_3, DMF, 70–80 °C, 5 h; (vii) PPh_3, THF, 25–30 °C, 1 h, H_2O, 55–60 °C, 4 h; (viii) CS_2 solution, $ClCO_2Et$, Et3N, THF, 0→30 °C, 3–4 h; (ix) RNH_2, toluene/acetonitrile, 25–30 °C, 4–18 h.

in the developed countries in AIDS patients and immunocompromised individuals for the selection of the compounds possessing broad-spectrum activity.

The preliminary antimycobacterial activity of all the compounds was evaluated by the agar dilution assay [25] at three different concentrations (50, 25, and 12.5 μg ml^{-1}) against three reference strains of mycobacterium, i.e. *M. tuberculosis* $H_{37}Rv$ ATCC 27294, *M. avium* ATCC 49601 and *M. intracellulare* ATCC 13950. The active compounds were then assayed for determination of minimum inhibitory concentration (MIC) against a panel of mycobacterial cultures consisting of appropriate reference strains of three mycobacterial species, 18 clinical isolates representing the sensitive and resistant (either isoniazid/rifampicin or by both) strains of *M. tuberculosis* were included in the study. Control drugs isoniazid, and linezolid were included in each batch of test.

4. Results and discussion

The results of in vitro antimycobacterial activities (MIC) are summarized in Table 1. The antimycobacterial activity data given in Table 1 clearly show that compounds, **15**, **16**, **18**, and **19** having amino, 2-pyridyl, 1-pyrrolidinyl and 1-piperidinyl moieties, respectively, at ethyl side chain of thiourea end exhibited significant activity against *M. tuberculosis* $H_{37}Rv$ ATCC 27294 strain and sensitive clinical isolates of *M. tuberculosis*. These compounds had an almost similar range of MIC values; they differ only by onefold or twofold dilution. The in vitro antimycobacterial activities of these compounds (**15**, **16**, **18**, and **19**) were comparable to that of isoniazid and linezolid against *M. tuberculosis* $H_{37}Rv$ strain and sensitive clinical isolates of *M. tuberculosis*. Compound **15** showed activity against resistant strains of *M. tuberculosis* (MIC 2–4 μg ml^{-1}) also whereas isoniazid is not active against resistant strains of *M. tuberculosis*. When the morpholine group was substituted at ethyl side chain of thiourea (**20**), there was a complete loss of activity. Replacement of substituted ethyl group by cyclopropyl group at thiourea end led to the formation of compound **17**, which exhibited excellent antimycobacterial activity (in vitro) against drug resistant and sensitive clinical isolates of *M. tuberculosis*. The MIC value of compound **17** was superior than isoniazid against all these strains, whereas in comparison with linezolid, compound **17** showed better antimycobacterial activity against *M. tuberculosis* $H_{37}Rv$ strain and sensitive clinical isolates of *M. tuberculosis* and equivalent activity against resistant clinical isolates of *M. tuberculosis*. Rest of the compounds had either little or no activity.

5. Conclusions

A new series of oxazolidinone derivatives has been designed, synthesized and evaluated for antituberculosis activity against *M. tuberculosis* $H_{37}Rv$ and clinical isolates. Some of these compounds exhibited good potency and their in vitro activity against sensitive and resistant strains of *M. tuberculosis* were found to be equivalent or better than linezolid an oxazolidinone derivative and isoniazid, a well-known drug for tuberculosis. Compound **17** showed significant in vitro profile with excellent MIC value against drug resistant and sensitive clinical isolates of *M. tuberculosis*.

6. Experimental protocols

6.1. Chemistry

Melting points were determined in open capillaries on a Büchi B-545 melting point apparatus. Compounds were routinely checked for their purity on silica gel 60 F_{254} TLC plates and their spots were visualized by exposing them to iodine vapor, UV light or by spraying the plates with Dragendorff's or $KMnO_4$ reagents. ^{1}H-NMR spectra were recorded on Bruker Advance DRX 200 MHz instrument as solutions (in $CDCl_3$ or DMSO-d_6) using TMS as internal reference, and chemical shifts values are expressed in δ units. Mass spectra were run on

Table 1
Range of MIC values (μg ml^{-1}) of compounds **11**–**20** against $H_{37}Rv$ and clinical isolates of *Mycobacterium tuberculosis*[a]

Compound	R	m.p. (°C)	% yield	M. tb. I	M. tb. II		M. a.	M. i.
					Sensitive ($N = 9$)[b]	Resistant ($N = 9$)[b]		
11	NH_2	139–140	69	2	4–> 16	> 16	> 16	8
12	NHCO-4-pyridyl	199–200	66	8	8	8	> 16	8
13	$CH(Et)CH_2OH$	87–88	93	NA[c]	NA	NA	NA	NA
14	$CH_2CH(OEt)_2$	73–74	90	8	4–16	4–16	> 16	8
15	$CH_2CH_2NH_2$	85–86	79	1	1–4	2–4	8	8
16	CH_2CH_2-2-pyridyl	144–145	97	0.5	1–2	4–8	8	8
17	cyclopropyl	198–199	65	0.06	0.125–1.0	1–2	8	8
18	CH_2CH_2-pyrrolidine	85–86	92	1.0	2–4	4–8	16	16
19	CH_2CH_2-piperidine	82–83	89	0.5	0.5–1.0	8	4	8
20	CH_2CH_2-morpholine	107–108	96	NA	NA	NA	NA	NA
Isoniazid				0.25	0.12–0.25	8–> 16	> 16	8
Linezolid				0.5	0.25–0.5	1–2	> 16	8

[a] M. tb. I = *Mycobacterium tuberculosis* $H_{37}Rv$ ATCC 27294; M. tb. II = *Mycobacterium tuberculosis* Clinical isolates; M. a. = *M. avium* ATCC 49601; M. i. = *M. intracellulare* ATCC 13950.
[b] N = number of clinical isolates used per group.
[c] NA = not active in preliminary screening up to 50 μg ml^{-1}.

Applied Biosystems API 3000 instrument using direct inlet system under positive ion electrospray ionization source. Elemental analyses were carried out with a Perkin Elmer 2400 analyzer and the values found were within ±0.4% of theoretical values.

*6.1.1. 1-(2-Fluoro-4-nitro-phenyl)-1/2H-benzotriazole (**3**)*

To a solution of benzotriazole (**1**, 5.0 g, 42.02 mmol) in DMF (25 ml) was added K_2CO_3 (8.7 g, 63.04 mmol) and 1,2-difluoro-4-nitrobenzene (**2**, 7.34 g, 46.16 mmol) at 25–30 °C with stirring and the resulting reaction mixture was stirred for 7 h at same temperature. After the reaction was completed, the reaction mixture was poured onto crushed ice and stirred for 0.5 h. The solid separated out was filtered through suction, washed with water and dried under reduced pressure to yield **3**, as pale yellow solid (10.6 g, 98%), m.p. 160–161 °C. ^{1}H-NMR ($CDCl_3$): δ 8.25–8.41 (m, 3 H), 8.06–8.14 (m, 1 H), 7.58–7.68 (m, 3 H). MS: *m/z* 259 (M + 1).

*6.1.2. 4-Benzotriazol-1/2-yl-3-fluoro-phenylamine (**4**)*

A mixture of compound **3** (10.5 g, 40.69 mmol), HCO_2NH_4 (12.85 g, 204 mmol), 10% Pd–C (1.05 g) in THF (80 ml) and MeOH (20 ml) was stirred at 25–30 °C for 4 h. After completion of reaction, the resulting mixture was filtered through celite pad, washed with MeOH (20 ml). The filtrate was concentrated under reduced pressure, to give the amine **4**, as red solid (9.0 g, 97%), m.p. 144–145 °C. ^{1}H-NMR ($CDCl_3$): δ 8.03 and 8.07 (2s, 1 H), 7.85–7.90 (m, 1 H), 7.27–7.48 (m, 5 H), 4.02 (br s, 2 H). MS: *m/z* 229 (M + 1).

*6.1.3. (4-Benzotriazol-1/2-yl-3-fluoro-phenyl)-carbamic acid benzyl ester (**5**)*

To a cooled (0 °C) solution of amine **4** (9.0 g, 39.47 mmol) in acetone (100 ml) and H_2O (50 ml) was added Na_2CO_3 (14.2 g, 134 mmol) and then benzyl chloroformate (11.4 g, 85% w/w, 67 mmol) over a period of 15 min. The mixture was allowed to warm to 25–30 °C and stirred for 4 h. After completion of reaction, the reaction mixture was poured onto ice-cold water (100 ml) and stirred for 30 min. Solid separated out was filtered through suction, washed with water and dried under reduced pressure to give **5**, as white solid (14.1 g, 98%), m.p. 127–128 °C. ^{1}H-NMR ($CDCl_3$): δ 8.04 & 8.08 (2s, 1 H), 7.86–7.88 (m, 1 H), 6.96–7.72 (m, 10 H), 5.18 (br s, 2 H). MS: *m/z* 363 (M + 1).

*6.1.4. (R)-3-(4-Benzotriazol-1/2-yl-3-fluoro-phenyl)-5-hydroxymethyl-oxazolidin-2-one (**6**)*

To a stirred solution of compound **5** (14.0 g, 38.67 mmol) in dry THF (140 ml) at –78 °C under N_2 was added drop-wise 1.6M *n*-butyllithium-hexane (3.46 g, 54 mmol). After 45 min, (*R*)-glycidyl butyrate (8.35 g, 58 mmol) was added and stirred at –78 °C for 1 h. Then the reaction mixture was allowed to warm to 25–30 °C and stirred for 12 h. After completion of reaction, aqueous solution of NH_4Cl (10 ml) was added to the reaction mixture and stirred for 15 min. Organic layer was separated and aqueous layer was extracted with EtOAc (1 × 30 ml). The combined organic layers were washed with H_2O (1 × 20 ml), brine (1 × 20 ml), dried (Na_2SO_4) and filtered. The filtrate was concentrated under reduced pressure to yield the crude product, which was purified by column chromatography over silica gel (100–200 mesh) using EtOAc/hexane (4:1) as eluent to give **6**, as a pale yellow solid (4.0 g, 32%), m.p. 181–182 °C. ^{1}H-NMR ($CDCl_3$): δ 7.95–8.10 (m, 1 H), 7.84–7.92 (m, 1 H), 7.55–7.65 (m, 1 H), 7.34–7.46 (m, 4 H), 4.72–4.81 (m, 1 H), 3.95–4.14 (m, 2 H), 3.54–3.87 (m, 2 H). MS: *m/z* 329 (M + 1).

*6.1.5. Methanesulfonic acid (R)-3-(4-benzotriazol-1/2-yl-3-fluoro-phenyl)-2-oxo-oxazolidin-5-ylmethyl ester (**7**)*

To a stirred solution of compound **6** (4.0 g, 12.2 mmol) and Et_3N (6.16 g, 61 mmol) in DCM (40 ml) was added drop-wise $MeSO_2Cl$ (3.5 g, 31 mmol) with stirring at 0 °C. The reaction mixture was then allowed to warm at 25–30 °C and stirred for 4 h at same temperature. After completion of reaction, aqueous solution of Na_2CO_3 (10 ml) was added to the reaction mixture and extracted with DCM (2 × 20 ml). The combined organic layer was dried (Na_2SO_4), filtered, and the filtrate was concentrated under reduced pressure to give **7**, as yellow solid (4.9 g, 99%), m.p. 121–123 °C. ^{1}H-NMR ($CDCl_3$): δ 8.10 and 8.05 (2s, 1 H), 7.77 and 7.83 (2d, J = 2.2 Hz, 1 H), 7.65 (t, J = 9.0 Hz, 1 H), 7.33–7.49 (m, 4 H), 4.92–4.96 (m, 1 H), 4.43–4.48 (m, 2 H), 4.20 (t, J = 9.0 Hz, 1 H), 3.96–4.04 (m, 1 H), 3.06 (2s, 3 H). MS: *m/z* 407 (M + 1).

*6.1.6. (R)-5-Azidomethyl-3-(4-benzotriazol-1/2-yl-3-fluoro-phenyl)-oxazolidin-2-one (**8**)*

A mixture of mesylate **7** (4.9 g, 12.07 mmol) and NaN_3 (1.17 g, 18 mol) in DMF (25 ml) was heated at 70–80 °C for 5 h. The mixture was poured into H_2O and filtered, the precipitate was air dried to give azide **8**, as pale yellow solid (4.0 g, 94%), m.p. 113–115 °C. ^{1}H-NMR ($CDCl_3$): δ 8.06 and 8.10 (2s, 1 H), 7.85 and 7.78 (2d, J = 2.4 Hz, 1 H), 7.64 (t, J = 9.0 Hz, 1 H), 7.32–7.49 (m, 4 H), 4.78–4.86 (m, 1 H), 4.12 (t, J = 9.0 Hz, 1H), 3.86–3.94 (m, 1 H), 3.53–3.78 (m, 2 H). MS: *m/z* 354 (M + 1).

*6.1.7. (R)-5-Aminomethyl-3-(4-benzotriazol-1/2-yl-3-fluoro-phenyl)-oxazolidin-2-one (**9**)*

To a solution of compound **8** (4.0 g, 11.33 mmol) in THF (40 ml), was added Ph_3P (4.46 g, 17.02 mmol) and resulting mixture was stirred at 25–30 °C for 1 h. To this, H_2O (1.0 ml) was added and the resulting reaction mixture was heated to 55–60 °C for 4 h. After completion of reaction, reaction mixture was concentrated under reduced pressure to give crude product. The compound was purified by column chromatography over silica gel (100–200 mesh) using 1% MeOH–$CHCl_3$ as eluent to give **9**, as a off white solid (1.7 g, 46%), m.p. 116–117 °C. ^{1}H-NMR (DMSO-d_6): δ 8.14 and 8.09 (2s, 1 H), 7.75–7.87 (m, 2 H), 7.41–7.58 (m, 4 H), 4.58–4.63 (m, 1 H), 4.10 (t, J = 9.0 Hz, 1 H), 3.86–3.93 (m, 1 H), 2.78 (t, J = 4.6 Hz, 2 H). MS: *m/z* 328 (M + 1).

*6.1.8. (R)-3-(4-Benzotriazol-1/2-yl-3-fluoro-phenyl)-5-isothiocyanatomethyl-oxazolidin-2-one (**10**)*

To a solution of amine **9** (0.8 g, 2.45 mmol) in dry THF (40 ml), was added Et_3N (0.24 g, 2.4 mmol) and cooled to 0 °C. To this, CS_2 solution (0.38 g, 5.0 mmol) was added and resulting reaction mixture was stirred at same temperature. After 3 h, $ClCO_2Et$ (0.26 g, 2.4 mmol) was added to the reaction mixture and stirred for another 1 h at 0 °C. After completion of reaction, reaction mixture was allowed to warm to 25–30 °C; H_2O (20 ml) was added to the mixture and extracted with EtOAc (1 × 20 ml), dried (Na_2SO_4) and filtered. The filtrate was concentrated under reduced pressure to give compound **10**, as white solid (0.88 g, 97%), m.p. 134–135 °C. ^{1}H-NMR ($CDCl_3$): δ 8.18 and 8.13 (2s, 1H), 7.85 and 7.91 (2d, J = 2.0 Hz, 1H), 7.73 (t, J = 10.0 Hz, 1H), 7.41–7.61 (m, 4H), 4.88–4.97 (m, 1H), 4.30 (t, J = 10.0 Hz, 1H), 3.86–4.11 (m, 3H). MS: *m/z* 370 (M + 1).

*6.1.9. General procedure for the synthesis of (R)-1-[3-(4-Benzotriazol-1/2-yl-3-fluoro-phenyl)-2-oxo-oxazolidin-5-ylmethyl]-3-substituted-thiourea (**11**–**20**)*

To a stirred solution of appropriate amine (RNH_2, 4.0 mmol) in toluene/acetonitrile (10 ml), was added 3-(4-benzotriazol-1/2-yl-3-fluoro-phenyl)-5-isothiocyanatomethyl-oxazolidin-2-one (**10**, 3.0 mmol) and stirred for 4–18 h at 25–30 °C. After completion of reaction, the reaction mixture was concentrated under reduced pressure to obtain crude product which was purified by column chromatography over silica gel (100–200 mesh) using 1% MeOH–$CHCl_3$ as eluent to give required product.

*6.1.9.1. (R)-1-[3-(4-Benzotriazol-1/2-yl-3-fluoro-phenyl)-2-oxo-oxazolidin-5-ylmethyl]-3-amino-thiourea (**11**)*. Compound **11** was obtained as white solid. ^{1}H-NMR (DMSO-d_6): δ 8.87 (br s, 1H), 8.12 (d, J = 8.0 Hz, 1H), 7.80 (t, J = 10.0 Hz, 2H), 7.44–7.55 (m, 5H), 4.80–5.00 (m, 1H), 4.48 (br s, 2H), 4.12 (t, J = 8.8 Hz, 1H), 3.91–4.05 (m, 1H), 3.80–3.90 (m, 2H). MS: *m/z* 402 (M + 1). Anal. Calcd for $C_{17}H_{16}FN_7O_2S$ (401.42): C, 50.86; H, 4.02; N, 24.43%. Found: C, 51.01; H, 3.88; N, 24.40%.

*6.1.9.2. (R)-1-[3-(4-Benzotriazol-1/2-yl-3-fluoro-phenyl)-2-oxo-oxazolidin-5-ylmethyl]-3-(isonicotinamide)-thiourea (**12**)*. Compound **12** was obtained as white solid. ^{1}H-NMR (DMSO-d_6): δ 8.54 (br s, 1H), 8.07 (m, 3H), 7.86 and 7.90 (2s, 2H), 7.19–7.80 (m, 5H), 4.80–4.90 (m, 1H), 3.60–4.10 (m, 7H). MS: *m/z* 507 (M +1). Anal. Calcd for $C_{23}H_{19}FN_8O_3S$ (506.51): C, 54.54; H, 3.78; N, 22.12%. Found: C, 54.61; H, 3.80; N, 22.44%.

*6.1.9.3. (R)-1-[3-(4-Benzotriazol-1/2-yl-3-fluoro-phenyl)-2-oxo-oxazolidin-5-ylmethyl]-3-(1-hydroxymethyl-propyl)-thiourea (**13**)*. Compound **13** was obtained as white solid. ^{1}H-NMR ($CDCl_3$): δ 8.16 and 8.12 (2s, 1H), 7.86 and 7.79 (2d, J = 2.0 Hz, 1H), 7.70 (t, J = 8.0 Hz, 2H), 7.39–7.60 (m, 5H), 6.30–6.60 (br s, 1H), 4.90–5.10 (m, 1H), 3.90–4.40 (m, 4H), 3.40–3.80 (m, 3H), 1.40–1.80 (m, 2H), 0.94 (t, J = 6.0 Hz, 3H). MS: *m/z* 459 (M + 1). Anal. Calcd for $C_{21}H_{23}FN_6O_3S$ (458.01): C, 55.01; H, 5.06; N, 18.33%. Found: C, 54.78; H, 4.99; N, 18.30%.

*6.1.9.4. (R)-1-[3-(4-Benzotriazol-1/2-yl-3-fluoro-phenyl)-2-oxo-oxazolidin-5-ylmethyl]-3-(2,2-diethoxy-ethyl)-thiourea (**14**)*. Compound **14** was obtained as white solid. ^{1}H-NMR ($CDCl_3$): δ 8.10 and 8.05 (2s, 1H), 7.82 and 7.76 (2d, J = 2.0 Hz, 1H), 7.63 (t, J = 8.0 Hz, 2H), 7.19–7.50 (m, 5H), 4.90–5.05 (m, 1H), 3.85–4.50 (m, 4H), 3.40–3.80 (m, 7H), 1.10–1.60 (t, J = 6.0 Hz, 6H). MS: *m/z* 503 (M + 1). Anal. Calcd for $C_{23}H_{27}FN_6O_4S$ (502.56): C, 54.97; H, 5.42; N, 16.72%. Found: C, 55.06; H, 5.49; N, 16.88%.

*6.1.9.5. (R)-1-(2-Amino-ethyl)-3-[3-(4-benzotriazol-1/2-yl-3-fluoro-phenyl)-2-oxo-oxazolidin-5-ylmethyl]-thiourea (**15**)*. Compound **15** was obtained as white solid. ^{1}H-NMR ($CDCl_3$): δ 8.12 and 8.05 (2s, 1H), 7.82 and 7.76 (2d, J = 2.0 Hz, 1H), 7.63 (t, J = 8.0 Hz, 2H), 7.19–7.50 (m, 5H), 4.85–5.00 (m, 1H), 3.85–4.50 (m, 4H), 3.45–3.80 (m, 4H), 2.21 (br s, 2H). MS: *m/z* 430 (M+1). Anal. Calcd for $C_{19}H_{20}FN_7O_2S$ (429.47): C, 53.14; H, 4.69; N, 22.83%. Found: C, 53.36; H, 4.41; N, 22.68%.

*6.1.9.6. (R)-1-[3-(4-Benzotriazol-1/2-yl-3-fluoro-phenyl)-2-oxo-oxazolidin-5-ylmethyl]-3-(2-pyridin-2-yl-ethyl)-thiourea (**16**)*. Compound **16** was obtained as white solid. ^{1}H-NMR ($CDCl_3$): δ 8.55 (br s, 1H), 8.17 and 8.13 (2s, 1H), 7.87 and 7.81 (2d, J = 2.0 Hz, 1H), 7.00–7.75 (m, 8H), 4.90–5.10 (m, 1H), 4.00–4.45 (m, 6H), 3.86 (br s, 2H), 3.06 (t, J = 6.2 Hz, 2H). MS: *m/z* 492 (M + 1). Anal. Calcd for $C_{24}H_{22}FN_7O_2S$ (491.54): C, 58.64; H, 4.51; N, 19.95%. Found: C, 58.33; H, 4.49; N, 19.88%.

*6.1.9.7. (R)-1-[3-(4-Benzotriazol-1/2-yl-3-fluoro-phenyl)-2-oxo-oxazolidin-5-ylmethyl]-3-cyclopropyl-thiourea (**17**)*. Compound **17** was obtained as white solid. ^{1}H-NMR (DMSO-d_6): δ 8.24 and 8.20 (2s, 1H), 8.05–8.15 (m, 1H), 7.80–8.00 (m, 3H), 7.40–7.70 (m, 4H), 4.90–5.15 (m, 1H), 4.28 (t, J = 6.0 Hz, 1H), 3.70–4.20 (m, 4H), 0.80–0.90 (m, 2H), 0.60–0.40 (m, 2H). MS: *m/z* 427 (M+1). Anal. Calcd for $C_{20}H_{19}FN_6O_2S$ (426.47): C, 56.33; H, 4.49; N, 19.71%. Found: C, 56.58; H, 4.21; N, 19.70%.

*6.1.9.8. (R)-1-[3-(4-Benzotriazol-1/2-yl-3-fluoro-phenyl)-2-oxo-oxazolidin-5-ylmethyl]-3-(2-pyrrolidin-1-yl-ethyl)-thiourea (**18**)*. Compound **18** was obtained as white solid. ^{1}H-NMR ($CDCl_3$): δ 8.09 and 8.05 (2s, 1H), 7.84 and 7.78 (2d, J = 2.0 Hz, 1H), 7.62 (t, J = 8.4 Hz, 1H), 7.25–7.50 (m, 5H), 4.80–5.05 (m, 1H), 3.80–4.25 (m, 5H), 3.20–3.70 (br s, 2H), 2.30–3.10 (m, 6H), 1.89 (br s, 4H). MS: *m/z* 484 (M + 1). Anal. Calcd for $C_{23}H_{26}FN_7O_2S$ (483.56): C, 57.13; H, 5.42; N, 20.28%. Found: C, 56.99; H, 5.64; N, 20.19%.

*6.1.9.9. (R)-1-[3-(4-Benzotriazol-1/2-yl-3-fluoro-phenyl)-2-oxo-oxazolidin-5-ylmethyl]-3-(2-piperidin-1-yl-ethyl)-thiourea (**19**)*. Compound **19** was obtained as white solid. ^{1}H-NMR ($CDCl_3$): δ 8.10 and 8.06 (2s, 1H), 7.85 and 7.79 (2d, J = 2.0 Hz, 1H), 7.62 (t, J = 8.4 Hz, 1H), 7.25–7.50 (m, 5H), 4.80–5.00 (m, 1H), 3.80–4.30 (m, 5H), 2.40–2.80 (m, 8H), 1.30–2.00 (m, 6H). MS: *m/z* 498 (M + 1). Anal. Calcd for $C_{24}H_{28}FN_7O_2S$

(497.59): C, 57.93; H, 5.67; N, 19.70%. Found: C, 58.03; H, 5.96; N, 20.01%.

*6.1.9.10. (R)-1-[3-(4-Benzotriazol-1/2-yl-3-fluoro-phenyl)-2-oxo-oxazolidin-5-ylmethyl]-3-(2-morpholin-4-yl-ethyl)-thiourea (**20**).* Compound **20** was obtained as white solid. ^{1}H-NMR ($CDCl_3$): δ 8.17 and 8.13 (2s, 1H), 7.90 and 7.83 (2d, $J = 2.0$ Hz, 1H), 7.70 (t, $J = 8.6$ Hz, 1H), 7.30–7.60 (m, 5H), 6.60 (br s, 1H), 4.90–5.10 (m, 1H), 3.95–4.45 (m, 4H), 3.77 (br s, 2H), 3.15–3.65 (m, 2H), 2.30–2.75 (m, 8H). MS: *m/z* 500 (M + 1). Anal. Calcd for $C_{23}H_{26}FN_7O_3S$ (499.56): C, 55.30; H, 5.25; N, 19.63%. Found: C, 55.11; H, 5.19; N, 19.50%.

6.2. Microbiology

The compounds were evaluated for antituberculosis activity by in vitro growth inhibition assay and agar dilution methods.

6.2.1. In vitro growth inhibition assay

The ability of the compounds to inhibit the growth of mycobacterium species was determined by agar diffusion assay. Briefly, reference strains *M. tuberculosis* $H_{37}Rv$ 27294 was grown in Middlebrook 7H9 broth containing 10% ADC supplement at 37 °C on a rotary shaker at 150 rpm for 7 days. The turbidity of the culture was adjusted to 0.5 McFarland. 0.50 ml of the individual cultures were then added to the molten Middlebrook 7H10 in 150 mm Petri plates. Uniform holes were then made in the media in which the three different concentrations (50, 25 and 12.5 μg ml^{-1}) of individual compounds were added. The plates were then incubated at 37 °C for 21–28 days. Compounds showing zone of inhibition greater or equal to the control drugs were considered active.

6.2.2. In vitro agar dilution assay

Minimum inhibitory concentration (MIC in μg ml^{-1}) against *M. tuberculosis* strains in agar dilution assay as per the NCCLS-M24-T2 recommendations [25]. The compounds and control drugs were dissolved in DMSO and diluted twofold to obtain 10 serial dilutions of each compound. Appropriate volumes of compounds were incorporated into duplicate plates of Middlebrook 7H10 agar medium supplemented with 10% Middlebrook supplement oleic acid–albumin–dextrose (OADC) enrichment at concentration of 0.03–16 μg ml^{-1}. Test organisms (mycobacterium strains) were grown in Middlebrook 7H9 broth containing 0.05% Tween 80 and 10% OADC supplement. After 7 days of incubation at 37 °C the broths were adjusted to the turbidity of 1.0 McFarland standard; the organism were further diluted 10-fold in sterile water containing 0.10% Tween 80. The resulting mycobacterial suspensions were spotted (3–5 μl per spot) onto 7H10 media plates containing different dilution of compounds/control drugs. The plates were sealed and incubated at 37 °C for 3–4 weeks in upright position. The MIC was recorded as the lowest concentration/highest dilution of the compounds/control drugs that completely inhibited the growth of mycobacterial cultures.

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