ISSN: 2249 –4820

Chemistry & Biology Interface, 2013, 3, 2, 107-115

Chemistry & Biology Interface

An official Journal of ISCB, Journal homepage; www.cbijournal.com

Research Paper

Synthesis And Antitubercular Activity of 1,3,4-Oxadiazoles Clubbed With Pyrroles

Kuldip Upadhyay[a], Atul Manvar[b], Ravi Chaniyara[c], Chetna Rajyaguru[d], Kena Raval[e], Hardevsinh Vala[f], and Anamik Shah[f]

[a] *Department of Chemistry, Regional Forensic Science Laboratory, Rajkot-360005, Gujarat, India.*
[b]*Centre for Synthesis & Chemical Biology, School of Chemistry & Chemical Biology, University College Dublin, Belfield, Dublin 4, Ireland.*
[c]*New Drug Discovery Division, Wockhardt Research Centre, Aurangabad-431136, Maharashtra, India.*
[d]*Department of Microbiology, Shri M. V. Mahila College, Rajkot-360005, India.*
[e]*Alembic Research Centre, Alembic Pharmaceuticals Limited, Gujarat, Vadodara 390003, India.*
[f]*National Facility for Drug Discovery (NFDD), Department of Chemistry, Saurashtra University, Rajkot-360005, Gujarat, India.*
Received 1 January 2013; Accepted 20 March 2013

Keywords: 1,3,4-Oxadiazole, Pyrrole, *M. tuberculosis*, *in vitro*

Abstract: *Mycobacterium tuberculosis* causes a serious health problem globally. In the present study, we enlighten the synthesis of molecularly diverse 1,3,4-oxadiazole in conjunction with substituted 1*H*-pyrrole motif. The resulting structural diversity was screened for their *in vitro* antitubercular activity against *Mycobacterium tuberculosis* H_{37}Rv strains. The analogs, such as **2c, 2i, 2k,** and **2m** were found promising against H_{37}Rv strains up to <6.25 µM MIC value. However, further exploration and modification are essential in a search for drug like *hint*.

Introduction

Mycobacterium tuberculosis is a leading epidemic and second most infectious disease after AIDS [1]. According to WHO report, about one third of the populations will be affected due to tuberculosis by 2020, if proper steps are not taken to control it [1]. At the present, TB can be treated by using the first-line and second-line antitubercular drugs. The fist-line treatment consisting 6-9 months course of four drugs in combination *e.g.* two months of pyrazinamide, ethambutol, isoniazid, and rifampin and latter 4-7 months of isoniazid and rifampin, whereas the second-line treatment consisting more expensive drugs, such as aminoglycosides, polypeptides, fluoroquinolones, and thioamides [2]. The drugs are classified as second-line drugs due to either of the reason, such as it may be less effective than the first-line drugs or having toxic side-effects or unavailable in developing countries [3]. Due to limited efficacy, the lengthy treatment schedule may lead to more expensive and extremely difficult to treat multi-drug resistance tuberculosis (MDR-TB) and extensively-drug resistance tuberculosis (XDR-TB) [4].

Corresponding Author* Tel/fax: +91 281 2589609/2581013. Email: anamik_shah@hotmail.com

ISSN: 2249 –4820

Chemistry & Biology Interface, 2013, 3, 2, 107-115

The treatment schedule for MDR-TB is typically consisting 18-24 months of second-line drugs in combination [5]. The most populous countries, India and China having more than 50% MDR-TB patients in the world [6]. Very recently, XDR-TB is defined as MDR-TB resistant to any fluoroquinolone or at least one second-line drugs [7]. There are several adverse effects caused by MDR-TB or XDR-TB drugs, such as dysglycaemia with gatifloxacin, hepatotoxicity with ethionamide, nephrotoxicity and ototoxicity with aminoglycosides [8]. Moreover, the AIDS pandemic has led to an explosion of HIV/TB co-infection for the patients living with HIV/AIDS [9]. Thus, there is a pressing need to investigate a new chemical prototype which could be administered with antireteroviral drugs for HIV/TB co-infections [10]. The current situation clearly represents to re-evaluate our approach towards antitubercular chemotherapy. The development of new clinical candidate encompasses novel modes of action against various targets is extremely necessitate [11]. Recognizing these serious facts, we probed a versatile chemical entities, such as coumarins [12], 1,4-dihydropyridines [13], tetrahydropyrimidines (patented) [14] , and more recently, a molecular hybrid of coumarins and 1,5-benzoazepines [15] as potent antituberculars.

Recently, in several reports the essential features of oxadiazole motif in medicinal and pesticide chemistry have been revealed (**Figure 1**). Predominantly, in drug discovery, oxadiazole is a significant pharmacophore, which is contributing for ligand binding [16]. Moreover, oxadiazole have also been employed as a bioisosteric replacement of carbonyl compounds, such as carbamates, hydroxamic esters, amides, and esters [16]. Particularly, the 1,3,4-oxadiazole pharmacophore have been screened for several therapeutic indications, for instance antitubercular [17], antibacterial [18], anticancer [19], antifungal [20], analgesic [21], anticonvulsant [22] *etc.* In addition, a molecular-hybrid of the drug pyrazinamide and 1,2,4-oxadiazole was identified as a potent antitubercular [23]. Furthermore, structurally diverse macrocyclic 1,3,4-oxadiazole was evaluated for antimycobacterial, antioxidant, and antibacterial activities [24]. In this light, recently, we have probed the 1,3,4-oxadiazole motif as a potent HIV non-nucleoside reverse transcriptase inhibitors (NNRTIs) (Unpublished results of NIH sponsored collaborative project with University of Medicine and Dentistry of New Jersey Medical School, NJ). Apart from oxadiazoles, various pyrrole derivatives were also considered as a promising 'lead' for antitubercular drug design [25]. In the present work, we have envision for the *in vitro* evaluation of structurally diverse 1,3,4-oxadiazole clubbed with 1*H*-pyrroles regiment for their antitubercular activity.

Experimental

Materials and methods

Chemicals and solvents were purchased from commercial sources. Melting points were determined in open capillary tubes with Electrothermal-9200 melting point apparatus and are uncorrected. Yields refer to isolated compounds, estimated to be >95% pure as determined by ^{1}H-NMR. TLC: Macherey-Nagel, TLC plates Alugram® Sil G/UV254. Detection under UV light at 254 nm. ^{1}H and ^{13}C-NMR spectra were recorded on Bruker Avance II 300 MHz and 75 MHz spectrometer respectively in DMSO-d_6. Chemical shifts (δ) are given in ppm relative to TMS, coupling constants (J) are in Hz. All IR

ISSN: 2249 –4820
Chemistry & Biology Interface, 2013, 3, 2, 107-115

spectra were recorded on Shimadzu FT-IR 8400 spectrometer using KBr pellet method. All ESI-MS spectra were recorded on JEOL SX 102/DA-6000. The elemental analyses of the compounds were carried out on Perkin Elmer 470R.

Synthesis of 4-acetyl-3,5-dimethyl-1*H*-pyrrole-2-carbohydrazide (1)

It was synthesized according to literature described procedure [26].

Representative procedure for synthesis of compounds 2a-n.

To a stirred mixture of compound **1** (0.01 mol) and corresponding carboxylic acids (0.01 mol), phosphorous oxychloride (5 equiv) was added under nitrogen atmosphere. The resulting mixture was heated to 100 ˚C in oil-bath for 4 h. After cooling the reaction mixture at ambient temperature, excess phosphorous oxychloride was evaporated *in vacuo.* The residue was quenched with water and extracted with ethyl acetate. The organic layer was washed with 20% sodium bicarbonate and brine solutions. The solvents were evaporated at reduced pressure, leaves the crude products. Finally, it was purified by crystallization in ethanol.

Spectral data of the compounds

1-[2,4-Dimethyl-5-(5-phenyl-1,3,4-oxadiazol-2-yl)-1*H*-pyrrol-3-yl]ethanone **(2a)**

Yield: 73%, mp (˚C): 192-194. IR (KBr, cm^{-1}): 3431, 3130, 3023, 1655, 1588. ^{1}H-NMR (300 MHz, DMSO-d_6) δ = 2.36 (s, 3H), 2.51 (s, 3H), 2.57 (s, 3H), 7.51-7.54 (m, 3H), 8.41-8.44 (m, 2H), 12.30 (brs, 1H). ^{13}C-NMR (75 MHz, DMSO-d_6) δ = 188.0, 166.7, 166.0, 137.4, 131.7, 131.0, 130.7, 130.1, 128.7, 122.3, 119.3, 31.2, 17.3, 12.5. Anal. Calcd for $C_{16}H_{15}N_3O_2$: C, 68.31; H, 5.37; N, 14.94. Found: C, 68.29; H, 5.40; N, 14.96. ESI-MS: *m/z* 282 $(M+H)^+$.

1-{5-[5-(2-Chlorophenyl)-1,3,4-oxadiazol-2-yl]-2,4-dimethyl-1*H*-pyrrol-3-yl}ethanone **(2b)**

Yield: 76%, mp (˚C): 206-208. IR (KBr, cm^{-1}): 3435, 3129, 3016, 1654, 1534. ^{1}H-NMR (300 MHz, DMSO-d_6) δ = 2.57 (s, 3H), 2.69 (s, 3H), 2.76 (s, 3H), 7.39-7.41 (m, 2H), 7.66 (t, *J* = 6.9, 2.1 Hz, 1H), 8.09 (t, *J* = 6.7, 2.3 Hz, 1H), 12.33 (brs, 1H). ^{13}C-NMR (75 MHz, DMSO-d_6) δ = 188.1, 167.8, 161.9, 137.9, 133.1, 131.1, 130.8, 130.1, 129.6, 129.0, 128.5, 122.7, 120.4, 31.6, 16.8, 12.2. Anal. Calcd for $C_{16}H_{14}ClN_3O_2$: C, 60.86; H, 4.47; N, 13.31. Found: C, 60.83; H, 4.45; N, 13.35. ESI-MS: *m/z* 316 $(M+H)^+$.

1-{5-[5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl]-2,4-dimethyl-1*H*-pyrrol-3-yl}ethanone **(2c)**

Yield: 67%, mp (˚C): 217-219. IR (KBr, cm^{-1}): 3419, 3129, 3015, 1650, 1581. ^{1}H-NMR (300 MHz, DMSO-d_6) δ = 2.54 (s, 3H), 2.67 (s, 3H), 2.73 (s, 3H), 7.83 (d, *J* = 6.0 Hz, 2H), 8.18 (d, *J* = 9.0 Hz, 2H), 12.35 (brs, 1H). ^{13}C-NMR (75 MHz, DMSO-d_6) δ = 187.6, 167.9, 165.8, 137.8, 135.4, 132.5, 131.8, 130.5, 129.7, 121.9, 119.0, 31.0, 17.8, 13.0. Anal. Calcd for $C_{16}H_{14}ClN_3O_2$: C, 60.86; H, 4.47; N, 13.31. Found: C, 60.85; H, 4.47; N, 13.34. ESI-MS: *m/z* 316 $(M+H)^+$.

1-{5-[5-(4-Nitrophenyl)-1,3,4-oxadiazol-2-yl]-2,4-dimethyl-1*H*-pyrrol-3-yl}ethanone **(2d)**

Yield: 59%, mp (˚C): 196-198. IR (KBr, cm^{-1}): 3419, 3113, 3010, 1655, 1521. ^{1}H-NMR (300 MHz, DMSO-d_6) δ = 2.39 (s, 3H), 2.53 (s, 3H), 2.60 (s, 3H), 8.27 (d, *J* = 9.0 Hz, 2H), 8.43 (d, *J* = 9.0 Hz, 2H), 12.28 (brs, 1H). ^{13}C-NMR (75 MHz, DMSO-d_6) δ = 187.2, 168.1, 167.3, 151.4, 137.9, 132.9,

ISSN: 2249 –4820
Chemistry & Biology Interface, 2013, 3, 2, 107-115

131.5, 130.9, 129.0, 122.8, 120.1, 31.8, 17.0, 12.1. Anal. Calcd for $C_{16}H_{14}N_4O_4$: C, 58.89; H, 4.32; N, 17.17. Found: C, 58.95; H, 4.35; N, 17.14. ESI-MS: *m/z* 327 $(M+H)^+$.

1-{5-[5-(2-Hydroxyphenyl)-1,3,4-oxadiazol-2-yl]-2,4-dimethyl-1*H*-pyrrol-3-yl}ethanone **(2e)**

Yield: 61%, mp (˚C): 198-200. IR (KBr, cm^{-1}): 3404, 3128, 3008, 1654, 1530. ^{1}H-NMR (300 MHz, DMSO-d_6) δ = 2.57 (s, 3H), 2.69 (s, 3H), 2.76 (s, 3H), 7.19-7.34 (m, 3H), 7.83 (t, *J* = 6.5, 2.2 Hz, 1H), 8.09 (brs, 1H), 12.31 (brs, 1H). ^{13}C-NMR (75 MHz, DMSO-d_6) δ = 188.1, 166.3, 165.7, 158.6, 138.5, 133.4, 131.2, 126.3, 124.9, 121.9, 119.4, 120.8, 109.3, 33.5, 17.9, 12.1. Anal. Calcd for $C_{16}H_{15}N_3O_3$: C, 64.64; H, 5.09; N, 14.13. Found: C, 64.69; H, 5.07; N, 14.10. ESI-MS: *m/z* 298 $(M+H)^+$.

1-{5-[5-(4-Methoxyphenyl)-1,3,4-oxadiazol-2-yl]-2,4-dimethyl-1*H*-pyrrol-3-yl}ethanone **(2f)**

Yield: 78%, mp (˚C): 222-224. IR (KBr, cm^{-1}): 3436, 3134, 2925, 1617, 1582. ^{1}H-NMR (300 MHz, DMSO-d_6) δ = 2.38 (s, 3H), 2.51 (s, 3H), 2.57 (s, 3H), 3.84 (s, 3H), 7.14 (d, *J* = 6.0 Hz, 2H), 7.97 (d, *J* = 9.0 Hz, 2H), 12.14 (brs, 1H). ^{13}C-NMR (75 MHz, DMSO-d_6) δ = 188.1, 166.1, 165.9, 159.5, 137.8, 132.4, 131.2, 123.1, 119.9, 116.2, 115.3, 56.8, 31.6, 18.0, 13.1. Anal. Calcd for $C_{17}H_{17}N_3O_3$: C, 65.58; H, 5.50; N, 13.50. Found: C, 65.55; H, 5.48; N, 13.47. ESI-MS: *m/z* 312 $(M+H)^+$.

1-{2,4-Dimethyl-5-[5-(3-nitrophenyl)-1,3,4-oxadiazol-2-yl]-1*H*-pyrrol-3-yl}ethanone **(2g)**

Yield: 64%, mp (˚C): 158-160. IR (KBr, cm^{-1}): 3419, 3129, 3014, 1652, 1527. ^{1}H-NMR (300 MHz, DMSO-d_6) δ = 2.37 (s, 3H), 2.55 (s, 3H), 2.59 (s, 3H), 8.23-8.49 (m, 3H), 8.83 (d, *J* = 2.3 Hz, 1H), 12.32 (brs, 1H). ^{13}C-NMR (75 MHz, DMSO-d_6) δ = 187.9, 166.2, 165.2, 150.4, 138.1, 135.3, 132.1, 131.6, 130.9, 125.7, 123.6, 121.8, 120.3, 31.9, 18.5, 13.2. Anal. Calcd for $C_{16}H_{14}N_4O_4$: C, 58.89; H, 4.32; N, 17.17. Found: C, 58.87; H, 4.35; N, 17.19. ESI-MS: *m/z* 327 $(M+H)^+$.

1-{2,4-Dimethyl-5-[5-(*o*-tolyl)-1,3,4-oxadiazol-2-yl]-1*H*-pyrrol-3-yl}ethanone **(2h)**

Yield: 77%, mp (˚C): 216-218. IR (KBr, cm^{-1}): 3419, 3118, 3014, 1650, 1528. ^{1}H-NMR (300 MHz, DMSO-d_6) δ = 2.34 (s, 3H), 2.53 (s, 3H), 2.58 (s, 3H), 7.36-7.48 (m, 3H), 7.96 (t, *J* = 6.1, 1.8 Hz, 1H), 12.16 (brs, 1H). ^{13}C-NMR (75 MHz, DMSO-d_6) δ = 187.9, 166.1, 165.8, 138.5, 137.5, 132.6, 131.6, 130.6, 129.4, 128.5, 127.4, 125.8, 120.0, 32.8, 19.4, 18.1, 12.4. Anal. Calcd for $C_{17}H_{17}N_3O_2$: C, 69.14; H, 5.80; N, 14.23. Found: C, 69.18; H, 5.85; N, 14.26. ESI-MS: *m/z* 295 $(M+H)^+$.

1-{2,4-Dimethyl-5-[5-(*p*-tolyl)-1,3,4-oxadiazol-2-yl]-1H-pyrrol-3-yl}ethanone **(2i)**

Yield: 75%, mp (˚C): 263-265. IR (KBr, cm^{-1}): 3450, 3118, 3015, 1654, 1583. ^{1}H-NMR (300 MHz, DMSO-d_6) δ = 2.38 (s, 6H), 2.51 (d, 3H), 2.57 (s, 3H), 7.40 (d, *J* = 3.0 Hz, 2H), 7.93 (d, *J* = 6.0 Hz, 2H), 12.17 (brs, 1H). ^{13}C-NMR (75 MHz, DMSO-d_6) δ = 187.6, 165.7, 165.0, 132.4, 131.2, 130.9, 129.5, 128.1, 125.2, 122.9, 120.0, 31.0, 22.5, 18.1, 12.9. Anal. Calcd for $C_{17}H_{17}N_3O_2$: C, 69.14; H, 5.80; N, 14.23. Found: C, 69.20; H, 5.78; N, 14.28. ESI-MS: *m/z* 295 $(M+H)^+$.

1-{5-[5-(2-Hydroxynaphthalen-1-yl)-1,3,4-oxadiazol-2-yl]-2,4-dimethyl-1*H*-pyrrol-3-yl}ethanone **(2j)**

Yield: 73%, mp (˚C): 223-225. IR (KBr, cm^{-1}): 3402, 3137, 2924, 1660, 1587. ^{1}H-NMR (300 MHz, DMSO-d_6) δ = 2.54 (s, 3H), 2.67 (s, 3H), 2.77 (s, 3H), 7.16-8.12 (m, 6H), 8.19 (s, 1H), 12.33 (brs, 1H). ^{13}C-NMR (75

ISSN: 2249 –4820
Chemistry & Biology Interface, 2013, 3, 2, 107-115

MHz, DMSO-d_6) δ = 188.1, 166.5, 165.9, 161.3, 135.4, 132.3, 131.7, 130.7, 130.0, 129.2, 128.5, 127.8, 125.2, 123.3, 120.4, 119.8, 117.3, 33.6, 18.5, 13.4. Anal. Calcd for $C_{20}H_{17}N_3O_3$: C, 69.15; H, 4.93; N, 12.10. Found: C, 69.19; H, 4.97; N, 12.14. ESI-MS: *m/z* 348 $(M+H)^+$.

1-{5-[5-(1*H*-Indol-2-yl)-1,3,4-oxadiazol-2-yl]-2,4-dimethyl-1*H*-pyrrol-3-yl}ethanone **(2k)**
Yield: 67%, mp (˚C): 83-85. IR (KBr, cm^{-1}): 3402, 3130, 2923, 1655, 1515. ^{1}H-NMR (300 MHz, DMSO-d_6) δ = 2.41 (s, 3H), 2.57 (s, 3H), 2.59 (s, 3H), 6.70 (s, 1H), 7.11-7.59 (m, 4H), 9.92 (brs, 1H), 12.29 (brs, 1H). ^{13}C-NMR (75 MHz, DMSO-d_6) δ = 187.5, 166.4, 165.0, 136.5, 130.1, 131.2, 129.0, 125.1, 122.9, 121.9, 121.5, 120.1, 119.5, 113.4, 102.7, 33.5, 17.5, 13.1. Anal. Calcd for $C_{18}H_{16}N_4O_2$: C, 67.49; H, 5.03; N, 17.49. Found: C, 67.53; H, 5.08; N, 17.53. ESI-MS: *m/z* 321 $(M+H)^+$.

2-[5-(4-Acetyl-3,5-dimethyl-1*H*-pyrrol-2-yl)-1,3,4-oxadiazol-2-yl]phenyl acetate **(2l)**
Yield: 63%, mp (˚C): 149-151. IR (KBr, cm^{-1}): 3419, 3132, 2924, 1652, 1542. ^{1}H-NMR (300 MHz, DMSO-d_6) δ = 2.35 (s, 6H), 2.54 (s, 3H), 2.56 (s, 3H), 7.43-7.64 (m, 3H), 8.06 (t, *J* = 6.3, 2.1 Hz, 1H), 12.23 (brs, 1H). ^{13}C-NMR (75 MHz, DMSO-d_6) δ = 187.9, 171.4, 166.1, 165.3, 155.6, 139.6, 133.5, 132.2, 131.4, 128.4, 125.3, 124.6, 120.5, 119.4, 30.3, 21.1, 16.9, 12.5. Anal. Calcd for $C_{18}H_{17}N_3O_4$: C, 63.71; H, 5.05; N, 12.38. Found: C, 63.76; H, 5.09; N, 12.43. ESI-MS: *m/z* 340 $(M+H)^+$.

1-{5-[5-(4-Bromophenyl)-1,3,4-oxadiazol-2-yl]-2,4-dimethyl-1*H*-pyrrol-3-yl}ethanone **(2m)**
Yield: 74%, mp (˚C): 242-244. IR (KBr, cm^{-1}): 3404, 3136, 2923, 1650, 1542. ^{1}H-NMR (300 MHz, DMSO-d_6) δ = 2.55 (s, 3H), 2.70 (s, 3H), 2.75 (s, 3H), 7.89 (d, *J* = 6.2 Hz, 2H), 8.23 (d, *J* = 7.2 Hz, 2H), 12.33 (brs, 1H). ^{13}C-NMR (75 MHz, DMSO-d_6) δ = 187.3, 168.8, 165.9, 135.4, 132.4, 130.7, 130.2, 126.3, 124.5, 122.7, 118.3, 30.4, 17.8, 13.4. Anal. Calcd for $C_{16}H_{14}BrN_3O_2$: C, 53.35; H, 3.92; N, 11.67. Found: C, 53.39; H, 3.97; N, 11.71. ESI-MS: *m/z* 361 $(M+H)^+$.

1-{2,4-Dimethyl-5-[5-(2-(phenylamino)phenyl)-1,3,4-oxadiazol-2-yl]-1*H*-pyrrol-3-yl}ethanone **(2n)**
Yield: 57%, mp (˚C): 220-222. IR (KBr, cm^{-1}): 3419, 3129, 2924, 1637, 1561. ^{1}H-NMR (300 MHz, DMSO-d_6) δ = 2.34 (s, 3H), 2.37 (s, 3H), 2.67 (s, 3H), 6.77-7.33 (m, 8H), 7.88 (t, *J* = 6.7, 2.1 Hz, 1H), 8.87 (brs, 1H), 12.25 (brs, 1H). ^{13}C-NMR (75 MHz, DMSO-d_6) δ = 189.5, 167.4, 166.2, 147.5, 142.4, 133.3, 131.3, 130.2, 129.9, 129.5, 127.3, 122.3, 121.9, 120.5, 119.3, 118.2, 117.4, 31.2, 17.3, 12.5, Anal. Calcd for $C_{22}H_{20}N_4O_2$: C, 70.95; H, 5.41; N, 15.04. Found: C, 70.99; H, 5.48; N, 15.10. ESI-MS: *m/z* 373 $(M+H)^+$.

Antitubercular activity

Antitubercular activity was determined using the BACTEC 460 system as modified below. Stock solutions as test compounds were prepared in dimethylsulfoxide (DMSO) at 1 mg/mL and sterilized by passage through 0.22 µm PFTE filters (Millex-FG, Millepore, Bedford MA). Fifty micro liters was added to 4 mL radiometric $7H_{12}$ broth (BACTEC 12B; Bectron Dickinson Diagnostic Instrument system, Sparks, MD) to achieve a final concentration of 6.25 µg/mL. Controls received 50 µL DMSO. Rifampin (Sigma Chemicals Co., St. Louis, MO) was included as a positive drug control. Rifampin was solubilized and diluted in DMSO and added to BACTEC-12 broth to achieve a range of concentration for concentration of minimum inhibitory

ISSN: 2249 –4820

Chemistry & Biology Interface, 2013, 3, 2, 107-115

concentration (MIC, lowest concentration inhibiting 99% inhibition of the inoculums). *M. Tuberculosis* $H_{37}Rv$ (ACTT 27294; American type culture collection (Rockville, MD) was cultured at 37 °C on a rotary shaker in middle brook $7H_9$ broth (Difco Laboratories, Detroiet, MI) supplemented with 0.2 v/v glycerol and 0.05% v/v Tween 80 unit the culture turbidity achieved an optical density of 0.45-0.55 at 550 nm. Bacteria were pelleted by centrifugation, washed twice and resuspended in one fifth of the original volume in dulbecoo's phosphate buffered saline [PBS, Irvine Scientific, Santa Ana, (A)]. Large bacterial clumps were removed by passage through 8 μm filter (malgene, Rochester, NY) and aliquots were frozen at -80 °C. The cultures were showed and an appropriate dilution performed such that a BACTEC-12B broth containing the test compounds. An additional control vial was included which received a further 1:100 diluted inoculums (as well as 50mL DMSO) for use in calculating the MIC of the rifampicin, respectively by establishing procedures. Cultures were incubated at 37 °C and the Growth of Inhibition (GI) determined daily until control cultures achieved a GI of 999. Assays were usually completed in 5-8 days. Percent inhibition was defined as 1-(GI of test sample/GI of control) × 10. Minimum inhibitory concentration of compound effecting a reduction in daily change in GI, which was less than that observed with a 1:100 diluted control culture on day the later reached a GI of at least 30 [27].

Results and discussion

The multi-step synthetic strategy for the preparation of target compounds was started with Paal-Knorr synthesis for 1*H*-pyrrole derivative by refluxing 2,4-pentanedione in the presence of ethyl-2-amino-3-oxobutanoate in acetic acid. Subsequent hydrazinolysis with hydrazine hydrate (98%) in ethanol, afforded 4-acetyl-3,5-dimethyl-1*H*-pyrrole-2-carbohydrazide, **1** [26]. Followed by condensation of hydrazide **1** with corresponding carboxylic acid in phosphorous oxychloride, afforded easily accessible compounds **2a-n** in moderate to good yields (57-78%) **(Scheme 1)**. The aromatic acids bearing functionalities, for instance Cl, Br, NO_2, Me, OMe, OCOMe, and OH as well as sterically hindered and (hetero)aromatic acids were also compatible under the established reaction conditions. The resulting compounds were characterized by ^{1}H-NMR, ^{13}C-NMR, IR, Mass, and elemental analysis and were tested for their antimycobacterial activity against *M. tuberculosis* $H_{37}Rv$ strains, by employing the microplate alamar blue assay (MABA) at TAACF (USA). The drug rifampin was used as a positive control. The relationship between structure and activity (SAR) is summarized in **Table 1**. Since the compounds have average biological effects it seems that trying to predict SAR of this is very difficult. The parent compound **2a** has shown 56% growth inhibition against *Mtb* $H_{37}Rv$ strain and was found moderately active. On comparison of a set of halogenated analogs towards antimycobacterial activity, compounds bearing *ortho* chloro **2b** group in the ring A, has shown average activity. In contrast, dramatic increase in growth inhibition was observed for its bioisoster **2c**, whilst compound **2m** with *para* bromo substitution in the ring A, exerted compatible activity to compound **2c**. From these findings, we may conclude that, the effect of specific functional group is not only a basic requisite for antimycobacterial activity but its ring-orientation is also essential to design a potent antitubercular analog. To probe the influence of molecular diversity on antimycobacterial activity, we have introduced strong electron-withdrawing

ISSN: 2249 –4820

Chemistry & Biology Interface, 2013, 3, 2, 107-115

nitro group in the ring A, compounds **2d** and **2g** have shown moderate and comparable activity towards *Mtb* H_{37}Rv strains. Structural modifications with electron donating substituents also reflected remarkable influence on mycobacterial growth inhibition as illustrated by one and half-fold gain in activity between two regio-isomers **2i** and **2h**. The compound **2f** bearing electron-rich *para* methoxy in the ring A has exhibited good activity against *Mtb* strains. The molecule bearing hydrogen-bond donor hydroxy group at *ortho* position of the ring A, compound **2e** as well as the effect of *β*-naphthol ring attached with 1,3,4-oxadiazole ring, compound **2j** or placement of acetate group at *ortho* position in the ring A, compound **2l** were found inactive. However the impact of insertion of indole heterocycle, compound **2k** and diphenyl amine to the oxadiazole ring on the antimycobacterial activity, compound **2n**, was found noteworthy against *Mtb* H_{37}Rv. Thus, we may conclude that, the effect of sterically hindered substituents may forbid the orientation of the ring. Membrane permeability and bioavailability are most frequently associated with some basic molecular descriptors such as Log*P* (partition coefficient), molecular weight, or hydrogen bond donors and acceptors counts in the molecule. In a current work, number of rotatable bonds and Lipinski's 'rule of 5' were calculated for the compounds **2a-n**. The poor absorption or permeation is most likely when, there are more than 5 H-bond donors, the molecular weight is above 500, the Log*P* is above 5 and there are more than 10 H-bond acceptors. This criterion is widely used as a filter for drug-like properties. In our cases, despite low potency, none of the compounds violates Lipinski's parameters, making them potentially promising antitubercular agents. The Log*P* was observed in the range of 1.56-4.07.

Conclusions

In summary, the present work demonstrates the synthesis and preliminary *in vitro* evaluation of 1,3,4-oxadiazoles against *mycobacterium tuberculosis* H_{37}Rv strains. Except few, most of molecules were found potent against *Mtb* H_{37}Rv. The preliminary screening results are highly encouraging but not sufficient, hence more versatile scaffold design, particularly with heteroaromatic carboxylic acids are in progress. Moreover, the cell walls of mycobacteria are lipid-rich and it is thought that more lipophilic compounds would better penetrate through this cell wall. In order to investigate the relationship between lipophilicity and antimycobacterial activity among the designed conjugates, the syntheses of a large number of lipophilic analogs by *N*-alkylation of the pyrrole fragment and its 3D-QSAR study are in progress. The results will be disseminated in due course.

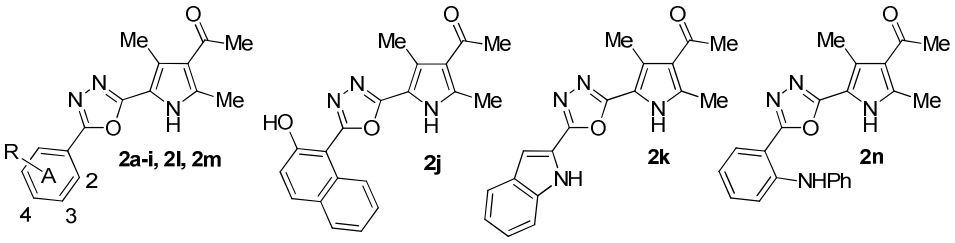
Acknowledgements

Financial support of the DST (India) under National Facility for Drug Discovery (NFDD) is gratefully acknowledged. The authors are also thankful to Dr. Cecil Kwong for antimycobacterial screening at Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF), USA.

ISSN: 2249 –4820

Chemistry & Biology Interface, 2013, 3, 2, 107-115

Table 1 Antitubercular activity of compounds **2a-n.**



Compds	R	[a]GI (%)	[b]Log*P*
2a	H	56	2.12
2b	2-Cl	36	2.89
2c	4-Cl	88	2.81
2d	4-NO_2	69	1.97
2e	2-OH	29	1.56
2f	4-OMe	70	2.18
2g	3-NO_2	66	1.90
2h	2-Me	54	2.44
2i	4-Me	84	2.58
2j	-	8	2.88
2k	-	81	1.96
2l	2-OCOMe	38	1.86
2m	4-Br	83	3.01
2n	-	70	4.07

[a](GI) Growth inhibition of virulent strain of *M. tuberculosis* at MIC <6.25 µg/mL. MIC of Rifampicin: 0.015–0.125 µg/mL against *M. tuberculosis* $H_{37}Rv$ (97% inhibition). [b]The Log*P* is calculated on MolSoft 2007.

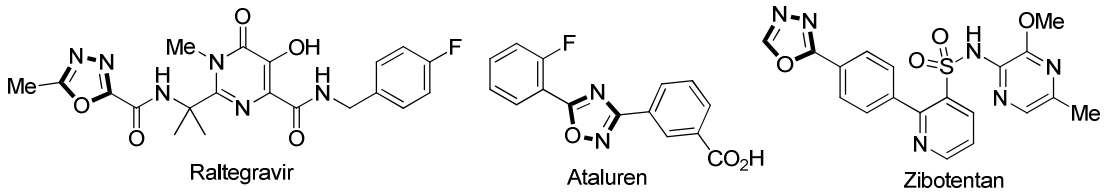
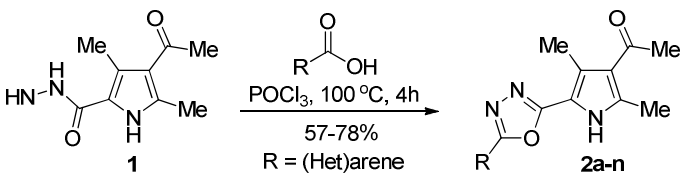


Figure 1 Oxadiazole motifs, Raltegravir (launched drug), Ataluren and Zibotentan (late stage clinical development).



Scheme 1 Synthesis of target compounds **2a-n**.

ISSN: 2249 –4820

Chemistry & Biology Interface, 2013, 3, 2, 107-115

References

[1] World Health Organization (WHO), global tuberculosis control **(2010)** http://www.who.int/tb/publications/global report/2010/en/index.html.

[2] Y.L. Janin, Bioorg. Med. Chem. **2007,** 15, 2479-2513 and references cited therein.

[3] A. Nayyar, R. Jain, Curr. Med. Chem. **2005,** 12, 1873-1886 and references cited therein.

[4] N.R. Gandhi, P. Nunn, K. Dheda, H.S. Schaaf, M. Zignol, D. Van Soolingen, P. Jensen, J. Bayona, The Lancet **2010,** 375, 1830-1843.

[5] W.W. Yew, C. Lange, C.C. Leung, Eur. Respir. J. **2011,** 37, 441-462.

[6] A. Koul, E. Arnoult, N. Lounis, J. Guillemont, K. Andries, Nature **2011,** 469, 483-490.

[7] L.G. Dover, G.D. Coxon, J. Med. Chem. **2011,** 54, 6157-6165 and references cited therein.

[8] W.W. Yew, M. Cynamon, Y. Zhang, Expert Opin. Emerging Drugs **2011,** 16, 1-21.

[9] B.R. Bloom, C.J. Murray, Science **1992,** 257, 1055-1064.

[10] R.P. Tripathi, N. Tewari, N. Dwivedi, V.K. Tiwari, Med. Res. Rev. **2005,** 25, 93-131.

[11] P.E.A. Da Silva, J.C. Palomino, J. Antimicrob. Chemother. **2011,** 66, 1417-1430.

[12] (a) A. Manvar, A. Malde, J. Verma, V. Virsodia, A. Mishra, K. Upadhyay, H. Acharya, E.C. Coutinho, A. Shah, Eur. J. Med. Chem. **2008,** 43, 2395-2403, (b) V. Virsodia, M.S. Shaikh, A. Manvar, B. Desai, A. Parecha, R. Loriya, K. Dholariya, G. Patel, V. Vora, K. Upadhyay, K. Denish, A. Shah, E.C. Coutinho, Chem. Biol. Drug Des. **2010,** 76, 412-424.

[13] A.T. Manvar, R.R.S. Pissurlenkar, V.R. Virsodia, K.D. Upadhyay, D.R. Manvar, A.K. Mishra, H.D. Acharya, A.R. Parecha, C.D. Dholakia, A.K. Shah, E.C. Coutinho, Mol. Divers. **2010,** 14, 285-305.

[14] (a) V. Virsodia, R.R.S. Pissurlenkar, D. Manvar, C. Dholakia, P. Adlakha, A. Shah, E.C. Coutinho, Eur. J. Med. Chem. **2008,** 43, 2103-2115, (b) D. Sarkar, S. Sarkar, R.R. Joshi, V.M. Khedkar, R.R. Pissurlenkar, E.C. Coutinho, A.K. Shah, PCT Int Appl, **2011** WO 2011/151701 A1 20111208. (c) D. Sarkar, S. Sarkar, R.R. Joshi, V.M. Khedkar, R.R. Pissurlenkar, E.C. Coutinho, A.K. Shah, Indian Pat Appl IN **2012** 2010DE01244 A 20120302.

[15] K. Upadhyay, A. Manvar, K. Rawal, S. Joshi, Y. Naliapara, J. Trivedi, R. Chaniyara, A. Shah, Chem. Bio. Drug Des. **2012,** 79, 1003-1008.

[16] J. Boström, A. Hogner, A. Llinas, E. Wellner, A.T. Plowright, J. Med. Chem. **2012,** 55, 1817-1830.

[17] (a) R.L. Bakal, S.G. Gattani, Eur. J. Med. Chem. **2012,** 47, 278-282, (b) F. Macaev, G. Rusu, S. Pogrebnoi, A. Gudima, E. Stingaci, L. Vlad, N. Shvets, F. Kandemirli, A. Dimogloa, R. Reynolds, Bioorg. Med. Chem. **2005,** 13, 4842-4850.

[18] H.M. Hassaneena, S.M.S. Attab, N.M. Fawzy, F.A. Ahmed, A.G. Hegazi, F.A. Abdalla, A.H. Abd El Rahman, Arch. Pharm. Pharm. Med. Chem. **2002,** 6, 251-261.

[19] C. Guo, S. Kephart, M. Ornelas, J. Gonzalez, A. Linton, M. Pairish, A. Nagata, S. Greasley, J. Elleraas, N. Hosea, J. Engebretsen, A.N. Fanjul, Bioorg. Med. Chem. Lett. **2012,** 22, 1230-1236.

[20] O. Prakash, M. Kumar, R. Kumar, C. Sharma, K.R. Aneja, Eur. J. Med. Chem. **2010,** 45, 4252-4257.

[21] A. Almasirad, A. Shafiee, M. Abdollahi, A. Noeparast, N. Shahrokhinejad, N. Vousooghi, S.A. Tabatabai, R. Khorasani, Med. Chem. Res. **2011,** 20, 435-442.

[22] S.K. Kashaw, V. Gupta, V. Kashaw, P. Mishra, J.P. Stables, N.K. Jain, Med. Chem. Res. **2010,** 19, 250-261.

[23] M.H. Gezginci, A.R. Martin, S.G. Franzblau, J. Med. Chem. **2001,** 44, 1560-1563.

[24] (a) B.V. Kumar, H.S.B. Naik, D. Girija, N. Sharath, H.V. Sudeep, H.J. Hoskeri, Arch. Pharm. Chem. Life Sci. **2012,** 345, 240-249, (b) M.B. Patel, N.R. Modi, J.P. Raval, S.K. Menon, Org. Biomol. Chem. **2012,** 10, 1785-1794.

[25] M. Biava, G.C. Porretta, G. Poce, C. Battilocchio, S. Alfonso, A. De Logu, F. Manetti, M. Botta, Chem. Med. Chem. **2011,** 6, 593-599.

[26] A. Manvar, A. Bavishi, A. Radadiya, J. Patel, V. Vora, N. Dodia, K. Rawal, A. Shah, Bioorg. Med. Chem. Lett. **2011,** 21, 4728-4731.

[27] L.A. Collins, S.G. Franzblau, Antimicrob. Agents Chemother. **1997,** 41, 1004-1009.