

Synthesis, Antibacterial and Anti-tubercular Evaluation Of Some 1,3,4-Oxadiazole based Mannich Bases

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Abstract: A novel series of Mannich bases (**3a-3g**) of substituted 1,3,4-oxadiazole were synthesized and purified. They were evaluated for their antibacterial and antitubercular potential against some pathogens. Compounds **3c** and **3e** exhibited promising antibacterial and antitubercular activities even at lower concentrations.

Key words: 1,3,4-Oxadiazole, Mannich bases, Antitubercular Activity.

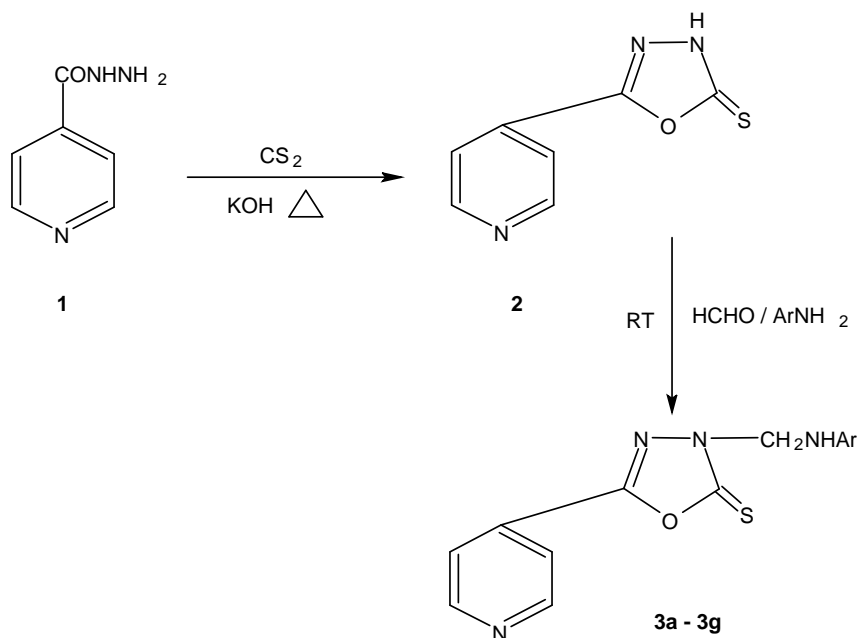
INTRODUCTION

Amongst all infectious diseases, Tuberculosis (TB) is one of the most common infectious diseases known to mankind¹. About one third of the world's current population is infected with *Mycobacterium tuberculosis*, and new infections occur at a rate of one per second². The proportion of people in the general population who become sick with tuberculosis each year is stable or falling worldwide but, because of population growth, the absolute number of new cases is still increasing³. The problem is due to the emergence of multi drug resistant (MDR) and extensively drug resistant (XDR) strains of *M. tuberculosis* and HIV infection, immigration, and globalization. Drug resistant TB is more difficult and more resistant to treat and more likely to be fatal⁴⁻⁵. Importantly, no new classes of anti-TB drugs with new mechanisms of action have been reported since last three decades.

The heterocyclic compounds containing nitrogen are well studied for their broad spectrum of activities⁶. Among them 1,3,4-oxadiazole and their derivatives constitute an important class of organic compounds which have been demonstrated to possess versatile activities including antibacterial⁷, antiviral⁸, antiinflammatory⁹, anticancer¹⁰, anti-HIV¹¹ antifungal¹² and promising anti-tubercular activity¹³. Over the past few decades, Mannich bases of heterocyclic molecules have found to show versatile pharmacological activities¹⁴⁻¹⁶. In

continuation of our interest in oxadiazole chemistry¹⁷⁻¹⁹, we herein report synthesis of some newer Mannich bases (**3a-3g**) and their evaluation for anti-tubercular and antibacterial activities.

In the present work, INH (**1**), an established anti-TB drug, was reacted with potassium hydroxide and carbon disulfide to yield 5-(pyridin-4-yl)-1,3,4-oxadiazole-2(3*H*)-thione (**2**), followed by treating it with various aromatic amines in the presence of formaldehyde to obtain desired Mannich bases (**3a-3g**).



Where,

Ar: **3a:** 3,4-Dichlorophenyl, **3b:** Phenyl, **3c:** 2,6-Dimethylphenyl, **3d:** 2,3-Dichloro phenyl, **3e:** 2-Nitrophenyl, **3f:** 2,6-Dichlorophenyl, **3g:** Phenyl carboxylic acid.

MATERIALS AND METHODS

Anti-tubercular Activity^{20, 21}

The antitubercular activity was carried out against *Mycobacterium tuberculosis* H₃₇Rv strain using Middle brook 7H-9 agar medium²². The agar medium containing compound, standard drug as well as DMSO (control) was inoculated with *Mycobacterium tuberculosis*. The inoculated bottles were incubated at 37°C for four weeks. At the end, they were checked for growth. Pyrazinamide and Streptomycin were used as standard drug at 10 µg/mL concentrations. The activity data is presented in Table I.

Antibacterial Activity²³

The antibacterial activity was performed *in vitro* against *E. coli* and *S. aureus* using agar-plate method. The MIC of these compounds was recorded as the lowest concentration of each compound in the plates with no visible growth of inoculated bacteria. Streptomycin was used as standard drug at 50 µg/mL concentration. The activity data is summarized in Table II.

Table I: Antitubercular activity of compounds (3a-3g):

Compounds	Activity ($\mu\text{g/mL}$)		
	25	50	100
3a	R	R	S
3b	R	S	S
3c	S	S	S
3d	S	S	S
3e	S	S	S
3f	R	R	S
3g	R	R	S
Rifampicin	S	S	S

R stands for resistant (inactive) and **S** stands for sensitive (active).

Table II: Anti-bacterial activity of titled compounds (3a-3g):

Compounds	Minimum Inhibitory Concentration (MIC) in $\mu\text{g/mL}$	
	<i>S. aureus</i>	<i>E. Coli.</i>
3a	300	250
3b	250	300
3c	100	150
3d	150	200
3e	100	250
3f	250	300
3g	350	400
Streptomycin	100	75

EXPERIMENTAL

Melting points were determined by open capillary methods on a 'Veego' VMP-D melting point apparatus and are uncorrected. TLC was done using silica gel G plates of size 3x8 cm (Sigma-Aldrich) and visualized by UV or in an iodine chamber. Column chromatography wherever necessary was performed on a neutral silica column (2.5 x 45 cm) using appropriate eluent. The IR spectra (KBr) were determined on FTIR 8400S, SHIMADZU spectrometer and the values are expressed in cm^{-1} . Mass spectra were recorded on Thermo Fisher Scientific Mass Spectrometry Instruments and ^1H NMR was recorded at 400 MHz in CDCl_3 using TMS as an internal reference standard.

Synthesis of 5-(pyridin-4-yl)-1,3,4-oxadiazole-2(3H)-thione (2):

To a solution of INH (**1**) (13.7g, 0.1 mol) in ethanol (100 mL) was added a solution of potassium hydroxide (5.6g, 0.1 mol) in water (36 mL) and stirred well. Carbon disulfide (7 mL) was then added and the mixture was refluxed till the evolution of H_2S is ceased. Excess of solvent was removed under vacuum and the residue was poured into ice-cold water (100 mL). It was filtered to remove suspended impurities and acidified with dil. HCl to obtain the desired product (88%), which was recrystallized from ethanol, mp: 238-242 $^\circ\text{C}$; IR (KBr, cm^{-1}): 3286 (NH *str.*), 3033, 2879 (-CH of pyridyl), 1625, 1500 (C=N *str.*), 1365 (C=S *str.*), 1141, 1108, 1064 (C-O-C, oxadiazole).

Synthesis of Mannich bases (3a-3g):

In a solution of thione (**2**) (2 g, 0.1 mol) in ethanol (20 mL), was added formaldehyde (2 mL, 0.11 mole), and appropriate amine (0.11 mole). The mixture was stirred for 8-10 h. at room temperature and left overnight as such. The product thus obtained was filtered and subjected to column chromatography (Ethyl acetate: Hexane; 1.5:3.5) to yield pure products. Following is the physicochemical characteristics and spectral data of titled compounds (3a-3g).

3-[(3,4-Dichlorophenyl)amino]methyl]-5-pyridin-4-yl-1,3,4-oxadiazole-2(3H)-

thione (3a): Mol. Formula $C_{14}H_{10}N_4OSCl_2$; Yield 85%; m.p. 88-90⁰C; IR (KBr, cm^{-1}): 3286 (NH *str.*), 3064 (Ar CH *str.*), 1595 (C=N *str.*), 1527 (NH *bend*), 1479 (C-N *str.*), 1413 (C=S *str.*), 1371, 1259 (C-N), 1130, 1085, 1026 (C-O-C oxadiazole), 993, 833, 761 (Ar CH *str.*), 692, 555 (C-Cl *bend*); ¹H NMR (ppm): 8.88 (s, 1H, NH), 7.50 (d, 2H,) pyridyl, 7.48 (d 2H,) pyridyl, [7.15 (s, 1H 1'-phenyl), 7.11 (d, 1H, 1'-phenyl), 7.08 (d, 1H, 2'-phenyl), 4.78(s, 2H, CH₂).

3-(Anilinomethyl)-5-pyridin-4-yl-1,3,4-oxadiazole-2(3H)-thione (3b): Mol. Formula $C_{14}H_{12}N_4OS$; Yield 74%; m.p. 92-95⁰C; IR (KBr, cm^{-1}): 3394 (NH *str.*), 3035 (Ar CH *str.*), 1598 (C=N *str.*), 1519 (NH *bend*), 1448 (C-N *str.*), 1380 (C=S *str.*), 1317, 1245 (C-N), 1178, 1066, 987 (C-O-C oxadiazole), 821, 750, 692 (Ar CH *str.*); ¹H NMR (ppm): 8.70 (s, 1H, NH), 7.90 (d, 2H,) pyridyl, 7.50-7.30 (d, 2H,) pyridyl, [7.26 (t, 1H), 7.17 (d, 2H), 6.95 (d, 2H)] phenyl, 4.09 (s, 2H, CH₂).

3-[(2,6-Dimethylphenyl)amino]methyl]-5-pyridin-4-yl-1,3,4-oxadiazole-2(3H)-

thione (3c): Mol. Formula $C_{16}H_{16}N_4OS$; Yield 88%; m.p. 165-170⁰C; IR (KBr, cm^{-1}): 3350, 3230 (NH *str.*), 2906 (Ar CH *str.*), 1620 (C=N *str.*), 1589, 1548 (NH *bend*), 1488, 1456 (C-N *str.*), 1402 (C=S *str.*), 1353, 1257 (C-N), 1157, 1093, 1045 (C-O-C oxadiazole), 835, 767, 692 (Ar CH *str.*); ¹H NMR (ppm): 8.79-8.77 (s, 1H, NH), 7.76 (d, 2H,) pyridyl, 7.74 (d, 2H,) pyridyl, [7.26 (d, 2H), 6.95 (t, 1H),] phenyl, 5.17 (s, 2H, CH₂), 2.17 (d, 6H, (CH₃)₂).

3-[(2,3-Dichlorophenyl)amino]methyl]-5-pyridin-4-yl-1,3,4-oxadiazole-2(3H)-

thione (3d): Mol. Formula $C_{14}H_{10}N_4OSCl_2$; Yield 76%; m.p. 129-130⁰C; IR (KBr, cm^{-1}): 3438 (NH *str.*), 3049 (Ar CH *str.*), 1589 (C=N *str.*), 1502 (NH *bend*), 1458 (C-N *str.*), 1415 (C=S *str.*), 1375, 1315 (C-N), 1253, 1151, 1085 (C-O-C oxadiazole), 1026, 952, 823 (Ar CH *str.*), 781, 690, 551 (C-Cl *bend*); ¹H NMR (ppm): 8.87 (s, 1H, NH), 7.60 (d, 2H,) pyridyl, 7.50 (d 2H,) pyridyl, [7.15 (d, 1H), 7.10 (t, 1H), 7.08 (d, 1H)] phenyl, 4.79(s, 2H CH₂).

3-[(2-Nitrophenyl) amino] methyl]-5-pyridin-4-yl-1,3,4-oxadiazole-2(3H)-

thione (3e): Mol. Formula $C_{14}H_{11}N_5O_3S$; Yield 70%; m.p. 180-182⁰C; IR (KBr, cm^{-1}): 3377 (NH *str.*), 3083 (Ar CH *str.*), 1616 (C=N *str.*), 1573 (NH *bend*), 1508 (C-N *str.*), 1427 (C=S *str.*), 1377, 1346 (C-N), 1161, 1091, 1033 (C-O-C oxadiazole), 860, 827, 742 (Ar CH *str.*); ¹H NMR (ppm): 8.71 (s, 1H, NH), 7.90 (d, 2H,) pyridyl, 7.60-7.50 (d, 2H,) pyridyl, [7.36 (t, 1H), 7.27 (d, 2H), 6.95 (d, 1H)] phenyl, 4.09 (s, 2H, CH₂).

3-[(2,6-Dichlorophenyl)amino]methyl]-5-pyridin-4-yl-1,3,4-oxadiazole-2(3H)-

thione (3f): Mol. Formula $C_{14}H_{10}N_4OSCl_2$; Yield 80%; m.p. 133-135⁰C; IR (KBr, cm^{-1}): 3126 (NH *str.*), 2856 (Ar CH *str.*), 1595 (C=N *str.*), 1544 (NH *bend*), 1460 (C-N *str.*), 1425 (C=S *str.*), 1382, 1267 (C-N), 1128, 1093, 1033 (C-O-C oxadiazole), 904, 827, 757 (Ar CH *str.*), 694, 561 (C-Cl *bend*); MS: m/e (rel. Intensity %): 356 (M⁺+3, 45%), 282 (M⁺-71, 10%), 180 (M⁺+1, 100%), 176, 174.

4-[(5-pyridin-4-yl-2-thioxo-1,3,4-oxadiazol-3(2H)yl)methyl]amino}benzoic acid (3g): Mol. Formula $C_{15}H_{12}N_4O_3S$; Yield 82%; m. p. 124⁰C; IR (KBr, cm^{-1}): 3218 (NH *str.*), 3074 (Ar CH *str.*), 1670, 1596 (C=N *str.*), 1514 (NH *bend*), 1429 (C-N *str.*), 1379 (C=S *str.*), 1290, 1234, 1164 (C-N), 1128, 1078, 1035 (C-O-C oxadiazole), 952, 862, 827, 757 (Ar CH *str.*).

RESULTS AND DISCUSSION

All the compounds were synthesized in quantitative yield and obtained in pure form through column chromatography, an outlined in the Scheme. The synthesized compounds were further evaluated for antibacterial and antitubercular activities.

The antitubercular screening showed that compounds **3c**, **3d** and **3e** were active against the mycobacterium, at the concentration of 25 µg/mL. The compound **3b** was active at the concentration of 50 µg/mL. However all the compounds were active at the concentration of 100 µg/mL. The antimicrobial activity suggested that MIC of these compounds was more than that of standard. However, compounds were moderately active against *S.*

aureus. Compounds **3c** and **3e** exhibited low MIC values (100 µg/mL) against *S. aureus*. But they exhibited weak to moderate activity against *E. coli*. Since the compounds exhibit promising activity, there is a need of further structural modification leading to better activity.

REFERENCES

1. Scheele S., Dolin P., Consensus statement. Global burden of tuberculosis, J. Am. Chem. Soc., 1999, 7, 677-686.
2. Nahid P., Pai M., Hopewell P., Advances in the diagnosis and treatment of tuberculosis, Proc. Am. Thorac. Soc., 2006, 3, 103-110.
3. Scientific Facts on Drug-resistant Tuberculosis, Green Facts, 2008, 12-18.
4. Barry C., Mizrahi V., Boshoff H., Effects of pyrazinamide on fatty acid synthesis by whole *Mycobacterial* cells and purified fatty acid synthase J. Bacteriol., 2002, 8, 2167-2172.
5. Yasir A., Jerald C., Advances in tuberculosis vaccine strategies, Nature Reviews Microbiology, 2006, 469-476.
6. Attimarad M., Rajasekran S., Rao G., Synthesis and anti-tuberculosis activity of some 5-phenyl-4-substituted amino-3-mercapto (4H), 1,2,4-triazoles, Ind. J. Pharm. Sci., 2000, 65, 475-477.
7. Ghattas A., El-Sherief H., Synthesis of some new heterocyclic 1,3,4-oxadiazoles with antibacterial activity, Pharmazie, 1982, 37, 410-412.
8. Shah H., Bhatt J., Desai N., Synthesis of 2,5-disubstituted 1,3,4-Oxadiazoles as potential antimicrobial, anticancer and anti-HIV agents, Ind. J. Chem., 1998, 37, 180-182.
9. Omar F., Mahfouz N., Rahman M., Design, synthesis and anti-inflammatory activity of some 1,3,4-oxadiazole derivatives, Eur. J. Med. Chem., 1996, 31, 819-825.
10. Aboraria A., Mahfouz N., El-Gendy M., Novel 5-(2-hydroxyphenyl)-3-substituted-2,3-dihydro-1,3,4-oxadiazole-2-thion derivatives: promising anticancer agents, Biorg. Med. Chem., 2006, 4, 1236-1246.
11. Shah H., Bhatt J., Desai N., Trivedi P., Synthesis of 2,5-disubstituted 1,3,4-oxadiazoles as potential antimicrobial, anticancer and anti-HIV agents, Ind. J. Chem., 1998, 37, 180-182.
12. Srivastava A., Pathak R., Bahel S., Synthesis and fungicidal activity of 5-substituted 1,3,4-oxadiazole-2-thiones and related compounds, J. Ind. Chem. Soc., 1990, 67, 606-607.
13. Shahar M., Siddiqui A. Ashraf Ali M., Synthesis and Anti-Tuberculostatic Activity of Novel 1,3,4-Oxadiazole Derivatives, J. Chinese Chem. Soc., 2007, 54, 5-8.
14. Vijayraghavan S., Somani R., Shirodkar P., Kadam V., Microwave assisted synthesis and antimicrobial activity of some newer Mannich bases, Int. J. Pharm.Tech. Res. 2009, 3, 811-815.
15. Joshi S., Khosla N., Tiwari P., Biorg., In vitro study of some medicinally important Mannich bases derived from antitubercular agent, Med. Chem., 2004, 12, 571-576.
16. Sriram D., Yogeswari P., Reddy S., Synthesis of pyrazinamide Mannich bases and its antitubercular properties, Biorg. Med. Chem., 2006, 16, 2113-2116.
17. Somani R., Shirodkar P., Synthesis, Antibacterial and Antitubercular evaluation of Some 1,3,4-Oxadiazole Analogues, Asian. J. Chem., 2008, 20, 6189-6194.
18. Shirodkar P., Somani R., Toraskar M., Kadam V., Synthesis and Biological Evaluation of 2,5-Disubstituted 1,3,4-Oxadiazole analogues, Ind. J. Pharm. Educ.Res., 2008, 42, 53-54.
19. Somani R., Shirodkar P., Kadam V., Synthesis and Antibacterial Activity of Some New 2,5-Disubstituted-1,3,4-oxadiazole Derivatives, Chinese J. Chem., 2008, 26, 1727-1731.
20. William M., Paul C., Elmer W., Stephen D.: Text book of Diagnostic Microbiology, Lippincot, 2002, 5, 935-937.
21. Dharmarajan S., Perumal Y., Sushma R., Synthesis of pyrazinamide Mannich bases and its antitubercular properties, Bioorg. Med. Chem., 2006, 16, 2113-2116.
22. Ellen J. B., Lancer R. P., Sydney M. F, Bailey and Scott's Diagnostic Microbiology, 2000, 9.
23. Barry A.: The Antimicrobial Susceptibility Test, Principle and Practice, Leu and Febiger, 1976, 64, 25783.
