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Anti-tubercular agents. Part IV: Synthesis and antimycobacterial evaluation of nitroheterocyclic-based 1,2,4-benzothiadiazines

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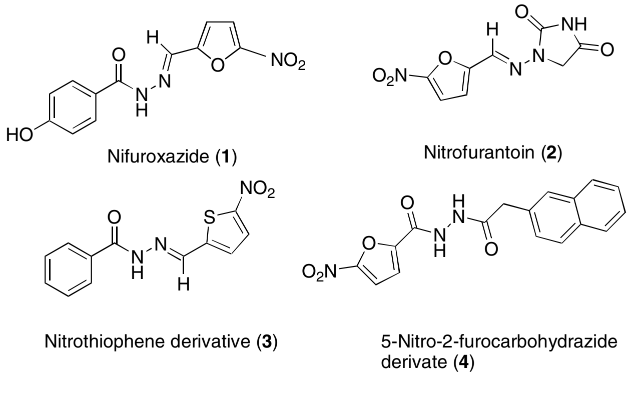
Abstract—In continuation of our earlier work on benzothiadiazines, we have prepared a series of nitrofuran, nitrothiophene and arylfuran coupled benzothiadiazines and evaluated them for antimycobacterial and antibacterial activities. One of the compounds **2f** has shown good in vitro antimycobacterial activity. All the synthesized compounds have shown moderate to good antibacterial activity.

Tuberculosis is a dreadful disease caused by bacteria called *Mycobacterium tuberculosis* and responsible for more human deaths than any other single infectious disease. It is estimated that, about one-third of the world's population is infected with this disease. According to World Health Organization, approximately 8 million people contact TB annually with almost 2 million deaths.[1] Furthermore, treatment of this disease caused by resistant bacteria is more difficult in immuno-compromised patients such as those infected with human immunodeficiency virus (HIV).[2] For these reasons, there is an overwhelming need to develop novel antimycobacterial agents with different mechanism of action aimed at a better understanding of antimycobacterial resistance.

The importance of nitrofurans and the isosteric nitrothiophenes as antimicrobial agents is well documented.[3] Although a lot of work has been done on these heterocycles, they still remained an active area of research interest.[4] The antimicrobial activity of nitroheterocycles (**1** and **2**, Fig. 1) is mainly due to the metabolic reduction of their nitro group by a class of enzymes called nitroreductases.[5] Recently, Lee and co-workers[6] have prepared a series of nitrofuranyl amides and tested for antimycobacterial activity. One of the analogues has shown excellent antimycobacterial activity, however, it did not reach the expected in vivo profile due to poor bioavailability. To overcome this problem, more recently, a new series



Nifuroxazide (**1**)

Nitrofurantoin (**2**)

Nitrothiophene derivative (**3**)

5-Nitro-2-furocarbohydrazide derivate (**4**)

Figure 1. Antibacterial (**1** and **2**), antimycobacterial (**3**) and antitrypanosomal (**4**) nitroheterocycles.

Keywords: Antimycobacterial; Antibacterial; Benzothiadiazines; Nitrofuran; Nitrothiophene; Arylfurans.

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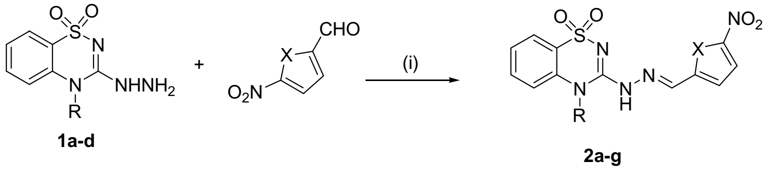
of these compounds have been prepared and evaluated for antimycobacterial activity. Some of these have shown much higher activity with improved bioavailability.[7] Similarly, 5-nitrothiophene derivatives have been studied as potential tuberculostatic agents (**3**, Fig. 1).[8] Further, these heterocycles have also shown significant bactericidal activity against growing and dormant *Mycobacterium bovis*.[9] Besides this, there has been considerable effort placed on these molecules as antitrypanosomal agents.[10] Davioud-Charvet and co-workers have identified that 5-nitro-2-furanocarbohydrazide as an excellent scaffold for the design of new antitrypanosomal agents (**4**, Fig. 1). [11] In addition to these properties, nitrofurans and nitrothiophenes have been described as antiamoebic,[12] hypoxia selective alkylating agents[13] and thioredoxin reductase inhibitors.[14] The related arylheterocyclic derivatives have also been found to exhibit different biological properties such as NHE-1,[15] *Escherichia coli* methionine aminopeptidase[16] and trypanosomal cysteine protease cruzain inhibitory activities.[17]

Sulfonamide derivatives have demonstrated potential antibacterial, anticancer, and anti-inflammatory activities in the literature[18] and benzothiadiazines can be considered as cyclic sulfonamide class of molecules. These compounds have been extensively studied as potassium channel openers.[19] Moreover, this ring system has also been known for antimicrobial activity.[20] Based on these findings, recently we have reported the synthesis and evaluation of antimycobacterial activity of these molecules by coupling with pyrazine carboxylic acid derivatives.[21] Keeping the importance of the nitroheterocycles in mind and in continuation of our studies on antimycobacterials particularly benzothiadiazines, herein we have reported the synthesis of 5-nitrofuran, 5-nitrothiophene and arylfuran coupled benzothiadiazines and evaluated them for their antimycobacterial and antibacterial activities.

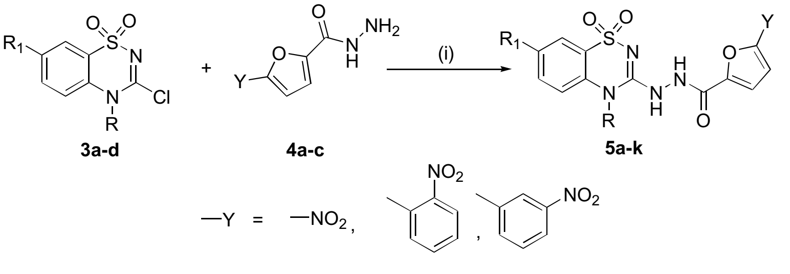
The preparation of desired compounds was carried out by the synthetic sequence illustrated in Schemes 1 and 2. The key intermediates **1a**–**d** and **3a**–**d** were prepared according to our previous report.[22] The desired compounds **2a**–**g** were obtained by the condensation of hydrazide derivatives (**1a**–**d**) with 5-nitrofurfural and 5-nitrothiophene aldehyde. The other target compounds **5a**–**k** were prepared by the reaction of chloro derivatives (**3a**–**d**) with carbohydrazide derivatives of aryl and nitrofurans (**4a**–**c**).[23] All the synthesized compounds were



1a: R = CH_3
1b: R = C_2H_5
1c: R = $CH(CH_3)_2$
1d: R = Ph

2a: R = CH_3, X = O
2b: R = CH_3, X = S
2c: R = C_2H_5, X = O
2d: R = C_2H_5, X = S
2e: R = $CH(CH_3)_2$, X = S
2f: R = Ph, X = O
2g: R = Ph, X = S

Scheme 1. Reagents and condition: (i) cat. acetic acid, ethanol, reflux.



3a: R = CH_3, R_1 = H
3b: R = C_2H_5, R_1 = H
3c: R = C_6H_5, R_1 = H
3d: R = CH_3, R_1 = Cl

4a: Y = NO_2
4b: 2-nitrophenyl
4c: 3-nitrophenyl
5a: R = CH_3, Y = NO_2, R_1 = H
5b: R= C_2H_5, Y= NO_2, R_1 = H
5c: R = Ph, Y = NO_2, R_1 = H
5d: R = CH_3 Y = NO_2, R_1 = Cl

5e: R = CH_3, Y = 2-nitrophenyl, R_1 = H
5f: R = C_2H_5, Y = 2-nitrophenyl, R_1 = H
5g: R = Ph, Y = 2-nitrophenyl, R_1 = H
5h: R = CH_3, Y = 2-nitrophenyl, R_1 = Cl
5i: R = CH_3, Y = 3-nitrophenyl, R_1 = H
5j: R = C_2H_5, Y = 3-nitrophenyl, R_1 = H
5k: R = Ph, Y = 3-nitrophenyl, R_1 = H

Scheme 2. Reagents and condition: (i) Et_3N, THF, rt, 24 h.

Table 1. Antibacterial and antimycobacterial activities of 3-benzothiadiazinyl hydrazone (**2a**–**g**) and hydrazide derivatives (**5a**–**k**) (MIC in μg/mL)

Compound	*M. tuberculosis* $H_{37}Rv$	*S. aureus*	*E. coli*	*P. aeruginosa*	*B. Subtilis*
2a	8	37.5	18.75	150	37.5
2b	8	37.5	37.5	150	18.75
2c	8	37.5	18.75	150	9.37
2d	16	18.75	18.75	150	18.75
2e	4	18.75	18.75	150	18.75
2f	1	75	37.5	150	37.5
2g	4	37.5	18.75	150	37.5
5a	32	37.5	18.75	150	18.75
5b	32	150	18.75	150	18.75
5c	8	37.5	18.75	150	75
5d	4	37.5	18.75	150	18.75
5e	>100	18.75	37.5	75	37.5
5f	>100	9.37	18.75	150	18.75
5g	>100	18.75	75	150	37.5
5h	>100	75	18.75	150	37.5
5i	>100	18.75	18.75	75	37.5
5j	>100	75	75	150	37.5
5k	>100	18.75	18.75	150	37.5
NF	—	18.75	18.75	150	18.75
RMP	0.12	—	—	—	—
INH	0.5	—	—	—	—

NF, Nitrofurantoin; RMP, Rifampicin; INH, Isoniazid.

characterized by ^1H NMR, IR and mass spectral analysis.[24]

The compounds **2a**–**g** and **5a**–**k** have been evaluated for the antimycobacterial activity and the results are summarized in Table 1. All compounds were initially screened against *M. tuberculosis* $H_{37}Rv$ at the single concentration of 100 μg/mL. The active compounds from this screening were further tested for minimum inhibitory concentration (MIC) determination using a broth macrodilution assay. Compounds demonstrating at least 90% inhibition in the primary screen were retested at lower concentrations by serial dilution against *M. tuberculosis* $H_{37}Rv$ to determine the actual MIC, using the nitrate reductase assay (NRA).[25] The growth in the microtitre plate is indicated by the change in colour to pink detected by the addition of NRA reagent. The MIC is defined as the lowest concentration of the compound showing no change in colour relative to controls. Rifampicin and isoniazid were used as reference drugs. All the hydrazone derivatives have shown activity between 1 and 16 μg/mL. Among these compounds nitrofuran-derived phenyl-substituted benzothiadiazine (**2f**) has shown good in vitro antimycobacterial activity (1 μg/mL). The replacement of furan ring with thiophene moiety in this compound has led to the reduction of activity by fourfold (**2g**). The replacement of phenyl group with alkyl groups has also reduced the effectiveness. Among the nitrofuran carbohydrazide derivatives, only 7-chloro-*N*-methyl (**5d**) substituted and *N*-phenyl (**5c**) derivatives have shown moderate activity. The corresponding nitrophenyl carbohydrazide derivatives have not shown any significant activity.

Further, these compounds (**2a**–**g** and **5a**–**k**) have also been evaluated for antibacterial activity by broth dilution method for minimum inhibitory concentration (MIC) as per the NCCLS[26] against clinical isolates of *Bacillus subtilis* MTCC 441, *Staphylococcus aureus* MTCC 96, *Escherichia coli* MTCC 443 and *Pseudomonas aeruginosa* MTCC 1688, and the results are summarized along with standard drug nitrofurantoin in Table 1. All the evaluated compounds have shown considerable in vitro activity against tested strains of organism except for *P. aeruginosa.*

In conclusion, we have synthesized a new series of nitroheterocyclic-coupled benzothiadiazines and evaluated them for their efficacy as antimicrobial agents. Compound **2f** was found to be most active against *M. tuberculosis* amongst the compounds tested. Furthermore, most of the compounds showed broad spectrum of activity against almost all bacterial strains screened, while these compounds exhibited mild activity against *P. aeruginosa.* Therefore, this work would be fruitful matrix for the development of a novel class of antimycobacterial agents.

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23. *Synthesis of compounds* **2a**–**g**. To the solution of compound (**1a**–**d**, 1 mmol) in ethanol (10 mL) were added 0.2 mL of acetic acid and 5-nitrofurfural or 5-nitrothiophene aldehyde (1.2 mmol). The resulting mixture was refluxed for about 8 h. The obtained suspension was filtered and washed with cold ethanol. The filtered compound was recrystallised from ethanol.

    Synthesis of compounds **5a**–**k**. To a stirred solution of carbohydrazide (**4a**–**c**, 1 mmol) and triethyl amine (0.17 mL, 1.22 mmol) in THF (10 mL) at room temperature was added a solution of 3-chloro-4-alkyl/aryl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**3a**–**c** and **3e**, 1 mmol) in THF (10 mL). The resulting mixture was stirred overnight at room temperature and then concentrated under reduced pressure. The residue thus obtained was dissolved in water (50 mL). The aqueous layer was extracted with ethyl acetate (4× 25 mL). The combined organic layer was washed with brine solution and dried over anhydrous Na_2SO_4. The resulting product was purified on column chromatography employing $CHCl_3/CH_3OH$ (9:1) as an eluant.
24. *Spectral data for selected compounds* **2a**: Yield 72%; mp 296–298 °C; ^1H NMR (200 MHz, DMSO-d_6): δ 11.80 (br s, 1H), 8.32 (s, 1H), 7.74–7.88 (m, 3H), 7.64 (d, $J = 8.05$ Hz, 1H), 7.50 (t, $J = 8.05$ Hz, 1H), 7.32 (d, $J = 3.66$ Hz, 1H), 3.68 (s, 3H); FAB MS $m/z = 349$ $(M)^+$; IR (KBr) (υ_{max}/cm^{-1}): 3231, 3101, 1586, 1552, 1353, 1134, 756, Anal. Calcd for $C_{13}H_{11}N_5O_5S$: C, 44.70; H, 3.17; N, 20.05. Found: C, 44.87; H, 3.08; N, 19.85.

    Compound **2f**: Yield 75%; mp >300 °C; ^1H NMR (200 MHz, DMSO-d_6): δ 11.55 (br s, 1H), 8.32 (s, 1H), 7.95 (d, $J = 8.25$ Hz, 1H), 7.75–7.90 (m, 4H), 7.44–7.72 (m, 4H), 7.25 (d, $J = 3.75$ Hz, 1H), 6.54 (d, $J = 8.25$ Hz, 1H); FAB MS $m/z = 411$ $(M)^+$; IR (KBr) (υ_{max}/cm^{-1}): 3320, 3060, 1615, 1566, 1545, 1495, 1350, 1140, 754. Anal. Calcd for $C_{18}H_{13}N_5O_5S$: C, 52.55; H, 3.18; N, 17.02. Found: C, 52.42; H, 3.09; N, 17.17.Compound **5d**: Yield 72%; ^1H NMR (200 MHz, DMSO-d_6): δ 11.05 (s, 1H), 10.08 (br s, 1H), 7.75 (d, $J = 1.87$ Hz, 1H), 7.62 (d, $J = 7.64$ Hz, 1H), 7.56 (d, $J = 3.89$ Hz, 1H), 7.42–7.44 (m, 1H), 7.24 (d, J = 3.89 Hz, 1H), 3.65 (s, 3H); ESI MS $m/z = 400$ $(M+1)^+$, 402 $(M+3)^+$; IR (KBr) (υ_{max}/cm^{-1}): 3386, 2923, 1675, 1539, 1483, 1353, 1282, 810, 671. Anal. Cald for $C_{13}H_{10}ClN_5O_6S$: C, 39.06; H, 2.52, N, 17.52. Found: C, 39.00; H, 2.77; N, 17.88.
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