Bioorganic & Medicinal Chemistry Letters 19 (2009) 1152–1154

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

5-Nitrofuran-2-yl derivatives: Synthesis and inhibitory activities against growing and dormant mycobacterium species

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ARTICLE INFO

Article history:
Received 18 November 2008
Revised 17 December 2008
Accepted 20 December 2008
Available online 25 December 2008

Keywords:
5-Nitrofuran-2-yl derivatives
Thiosemicarbazones
Tuberculosis
Dormant TB

ABSTRACT

Eighteen 5-nitrofuran-2-yl derivatives were prepared by reacting 5-nitro-2-furfural with various (sub)-phenyl/pyridyl thiosemicarbazide using microwave irradiation. The compounds were tested for their in vitro activity against tubercular and various non-tubercular mycobacterium species in log-phase and 6-week-starved cultures. Compound *N*-(3,5-dibromopyridin-2-yl)-2-((5-nitrofuran-2-yl)methylene)hydrazinecarbothioamide (**4r**) was found to be the most potent compound (MIC: 0.22 μM) and was 3 times more active than standard isoniazid (INH) and equally active as rifampicin (RIF) in log-phase culture of *Mycobacterium tuberculosis* H37Rv. In starved *M. tuberculosis* H37Rv, **4r** inhibited with MIC of 13.9 μM and was found to be 50 times more active than INH and slightly more active than RIF.

Mycobacterium tuberculosis (MTB) causes more human deaths than any other single infectious organism with an estimated eight million new tuberculosis (TB) cases and two million fatalities each year.[1] MTB has two features that render it the deadliest infectious disease to date: its high infectivity or virulence and its ability to enter latency for subsequent reactivation, a phenomenon that leads to deadliest synergy with acquired immune deficiency syndrome (AIDS).[2] As a result TB is also the leading cause of death for AIDS patients. Furthermore, the emergence of multi-drug resistance TB (MDR-TB) is severely hampering TB treatment,[3] and therefore is an *urgent need* to develop novel TB chemotherapeutics. A new TB treatment should offer at least one of three improvements over the existing regimens: (a) shorten the total duration of effective treatment and/or significantly reduce the total number of doses needed to be taken under DOTS supervision; (b) improve the treatment of MDR-TB, which cannot be treated with isoniazid (INH) and rifampin (RIF) and/or (c) provide more effective treatment of latent/dormant TB infection, which is essential for eliminating TB. Earlier 5-nitrofuranayl-2-amide derivative was reported for activity against MTB with minimum inhibitory concentration (MIC)[4] of 0.0002 μg/mL; and we have also reported various thiosemicarbazones with lowest MIC of 0.05 μg/mL.[5,6] Given the promising biological profile of 5-nitrofuranayl-2-amide derivative and thiosemicarbazones, we decided to design new derivatives by combining both the pharmacophores. In the present study, we report synthesis of eighteen 5-nitrofuran-2-yl derivatives and its in vitro activity against tubercular and various non-tubercular mycobacterium species in log-phase and 6-week-starved cultures.

The synthesis of 5-nitrofuran-2-yl derivatives was carried out in three steps, as shown in Scheme 1. First, to a solution of (sub)anilines/(sub)2-aminopyridines (**1a–r**) (0.01 mol) in ethanol (10 mL) was added potassium hydroxide (0.01 mol) and carbon disulfide (0.75 mL), and the mixture was stirred at 15–20 °C for 1 h to form a potassium salt of dithiocarbamate (**2a–r**). To the stirred mixture was added hydrazine hydrate (0.01 mol), and the stirring was continued at 80 °C for 1 h to obtain corresponding (sub)phenyl/pyridyl thiosemicarbazide (**3a–r**) in 90% yield. Thiosemicarbazide derivatives on condensation with 5-nitro-2-furfural in the presence of glacial acetic acid afforded various thiosemicarbazones (**4a–r**) (Table 1) in 62–86% yields. The reaction utilizes the micro wave irradiation in an unmodified domestic microwave oven at 80% intensity with 30 s/cycle for 3 min. The resultant solid was washed with dilute ethanol dried and recrystallized from ethanol–chloroform mixture. The purity of the compounds was checked by TLC and elemental analyses, and the compounds of this study were identified by spectral data. In the ^{1}H NMR spectra the signals of the respective protons of the prepared derivatives were verified on the basis of their chemical shifts, multiplicities, and coupling constants. The spectra of all the compounds showed a double doublets in the range of 7.55–7.56 δ ppm and 7.59–7.60 δ ppm corresponding to third and fourth position protons of the furan ring, a singlet at 7.43 δ ppm corresponding to methylene proton and a D_2O exchangeable singlet at 7.26 δ ppm corresponding to NH pro-

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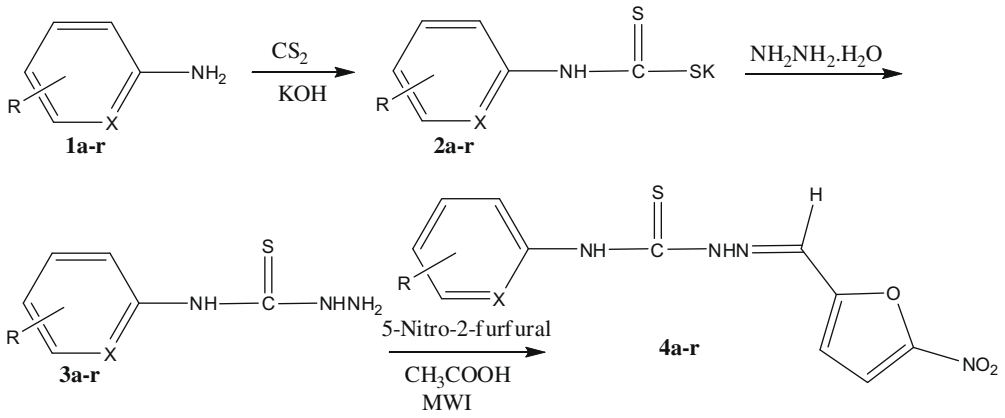
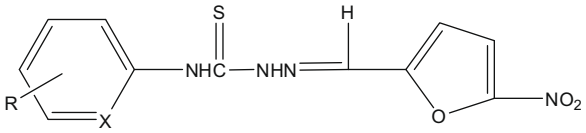
doi:10.1016/j.bmcl.2008.12.088



Scheme 1. Synthetic protocol of compounds **4a–r**.

Table 1
Physical constants and antimycobacterial activity



Compound	R	X	Yield (%)	MP (°C)	IC_{50}[a] (μM)	MIC in μM						
						MTB[b]	MS[c]	MM[d]	MF[e]	MP[f]	MV[g]	MK[h]
4a	H	H	59.8	138–140	>215.2	10.7	172.2	86.1	43.0	10.7	43.0	5.3
4b	4-CH_3	H	33.1	152–154	>205.4	5.1	41.0	20.5	41.0	2.5	41.0	20.5
4c	4-OCH_3	H	45.6	179–180	>195.1	9.7	39.0	78.0	39.0	4.85	78.0	39.0
4d	2-Br	H	87.9	160–162	>169.2	0.54	33.8	67.1	33.8	16.9	16.9	8.4
4e	4-Cl	H	90.9	152–155	>192.4	1.23	19.1	38.4	38.4	9.6	9.6	38.4
4f	4-SO_2 NH_2	H	88.6	154–155	<169.2	2.1	8.4	67.6	33.8	16.8	2.1	33.8
4g	2,4-$(CH_3)_2$	H	41.0	146–147	>196.3	2.45	314.1	78.5	39.2	4.9	39.2	9.8
4h	2,6-$(CH_3)_2$	H	76.9	185–186	>196.3	4.9	39.2	78.5	39.2	9.8	39.2	39.2
4i	2,5-$(CH_3)_2$	H	90.5	134–135	>196.3	1.2	157.0	157.0	78.5	4.8	2.4	78.5
4j	2,6-$(C_2H_5)_2$	H	53.8	150–151	>180.4	1.1	36.0	72.1	72.1	36.0	72.1	36.0
4k	2,4-$(OCH_3)_2$	H	83.7	197–200	>178.3	4.4	35.6	17.8	35.6	4.4	71.1	35.6
4l	2,4-$(NO_2)_2$	H	91.1	125–127	<164.3	0.52	16.4	32.8	65.1	32.8	65.1	32.8
4m	H	N	65.1	178–179	214.5	12.1	48.4	48.4	48.4	6.0	24.2	12.1
4n	3-CH_3	N	78.7	200–201	<204.7	5.1	20.5	10.2	10.2	10.2	20.5	5.1
4o	4-CH_3	N	78.9	195–197	<204.7	2.6	20.5	20.5	5.1	5.1	2.6	5.1
4p	5-Cl	N	83.6	204–205	191.8	1.2	2.4	38.3	38.3	38.3	4.8	4.8
4q	4,6-$(CH_3)_2$	N	91.8	199–200	<195.7	2.4	4.8	39.1	39.1	39.1	39.1	39.1
4r	3,5-Br_2	N	60.3	185–186	139.1	0.22	6.9	6.9	3.4	6.9	3.4	1.7
	Isoniazid				>455.8	0.66	45.57	22.82	22.82	91.15	182.3	182.3
	Rifampicin				>75.9	0.23	1.89	30.38	1.89	30.38	3.80	7.59
	Ciprofloxacin				>188.5	4.71	2.35	2.35	4.71	4.71	4.71	9.45

[a] Cytotoxicity in mammalian Vero cell lines.
[b] *M. tuberculosis*.
[c] *M. smegmatis*.
[d] *M. microti*.
[e] *M. fortuitum*.
[f] *M. phlei*.
[g] *M. vaccae*.
[h] *M. kansasii*.

tons. The elemental analysis results were within (0.4%) of the theoretical values.

The compounds were screened for their in vitro antimycobacterial activity against MTB, and non-tubercular mycobacterial (NTM) species like *M. smegmatis* ATCC 14468, *M. microti* MTCC 1727, *M. vaccae* MTCC 997, *M. phlei* MTCC 1724, *M. fortuitum* MTCC 951, and *M. kansasii* MTCC 3058 by agar dilution method similar to that recommended by the National Committee for Clinical Laboratory Standards[7] for the determination of MIC in triplicate. The minimum inhibitory concentration (MIC) is defined as the minimum concentration of compound required to give complete inhibition of bacterial growth. MICs of the synthesized compounds along with the standard drugs for comparison are reported (Table 1).

In the initial log-phase culture screening against MTB, the compounds showed good activity with MICs ranging from 0.22 to 12.1 μM. Three compounds (**4d, 4l**, and **4r**) showed excellent activity with MIC of <1 μM and were found to be more active than INH (MIC: 0.66 μM). One compound **4r** with MIC of 0.22 μM was found to be more active than rifampicin (RIF) (MIC: 0.23 μM). Twelve compounds were more potent than ciprofloxacin (MIC of 4.71 μM). Compound *N*-(3,5-dibromopyridin-2-yl)-2-((5-nitrofuran-2-yl)methylene)hydrazinecarbothioamide (**4r**) was found to

Table 2
Inhibitory activities of selected compounds against log-phase and 6-week-starved mycobacterial cultures

Compound	MIC in μM against MTB[a]		Compound	MIC in μM against MS[b]	
	Log-phase cells	Six-week-starved cells		Log-phase cells	Six-week-starved cells
4d	0.54	33.8	**4e**	19.1	133.7
4i	1.1	153.9	**4f**	8.4	75.6
4j	1.2	36.0	**4l**	16.4	213.2
4l	0.52	32.8	**4p**	2.4	43.2
4p	1.2	38.3	**4q**	4.8	91.2
4r	0.22	13.9	**4r**	6.9	124.2
INH	0.66	729.1	INH	45.57	>729.1
Rifampin	0.23	15.2	Rifampin	1.89	22.6

[a] *M. tuberculosis*.
[b] *M. smegmatis*.

be the most active compound in vitro with MICs of 0.22 μM against MTB. With respect to structure–MTB activity relationships, pyridylthiosemicarbazones were found to be more active than phenylthiosemicarbazones. Among the phenyl or pyridyl ring substituents, electron-withdrawing groups like nitro (**4l**), and halogen (**4d, 4e**, **4p**, and **4r**) enhanced the activity. Compounds with electron-donating groups decreased the activity considerably (**4b, 4c, 4g–k, 4n, 4o**, and **4q**). Among the methyl substituted phenyl- and pyridylthiosemicarbazones derivatives, the di-substituted compounds (**4g–j** and **4q**) showed enhanced activity compared with the mono-substituted derivative (**4b**, **4n**, and **4o**). All the compounds were also screened for atypical mycobacteria (AM), AM infection[8] an illness caused by a type of mycobacterium other than tuberculosis which cause a wide variety of infections such as abscesses, septic arthritis, and osteomyelitis. They can also infect the lungs, lymph nodes, gastrointestinal tract, skin, and soft tissues. The rate of AM infections is rare, but it is increasing as the AIDS population grows. Populations at risk include individuals who have lung disease and weakened immune systems. The synthesized compounds inhibited *M. smegmatis* (MS) with MICs ranging from 2.4 to 172.2 μM and 13 compounds were more potent than INH (MIC: 45.57 μM). With regard to activity against *M. micriotii* the compounds showed activity with MICs ranging from 6.9 to 157.0 μM and four compounds were more potent than INH (MIC: 22.82 μM). *M. vaccae* was inhibited by the synthesized compounds with MICs ranging from 2.1 to 78.0 μM and all 18 compounds were more potent than INH (MIC: 182.3 μM). All the compounds also inhibited *M. phlei* (MP) with MICs ranging from 2.5 to 39.1 μM and were more potent than INH (MIC: 91.15 μM). Against *M. fortuitum* the compounds showed activity with MICs ranging from 3.4 to 78.5 μM and three compounds were more potent than INH (MIC: 22.82 μM). The compounds were also screened against *M. kansasi* and were inhibited with MICs ranging from 1.7 to 39.2 μM and all compounds were more potent than INH (MIC: 182.3 μM). Compound **4r** inhibited all the eight mycobacterium species with MIC ranging from 0.22 to 6.9 μM and was more potent than INH.

The compounds which showed good activity against log-phase culture of MTB and MS were further screened against 6-week-starved cells of MTB and MS according to the literature procedure.[9] Against MTB, six compounds were tested and they inhibited starved culture of MTB with MICs ranging from 13.9 to 153.9 μM (Table 2). INH had poor activity against starved cells with MIC of 729.1 μM. As previously observed[9] RIF retained activity, although it is considerably less active against non-growing than against log-phase cells. All the six tested compounds were more potent than INH and one compound (**4r**) was found to be more potent (with MIC of 13.9 μM) than RIF (MIC: 15.2 μM). The presence of persistent and dormant MTB is thought to be the cause for the lengthy TB chemotherapy, since the current TB drugs are not effective in eliminating persistent or dormant bacilli. This study revealed that, these molecules active against slowly growing or non-growing persistent bacilli are thought to be important to achieve a shortened therapy. In the case of starved MS culture, the tested compounds inhibited with MIC values ranging from 43.2 to 213.2 μM and were more potent than INH (MIC: 729.1 μM). Compound *N*-(5-chloropyridin-2-yl)-2-((5-nitrofuran-2-yl)methylene)hydrazinecarbothioamide (**4p**) was found to be most active compound with MIC of 43.2 μM.

The compounds were further examined for toxicity (IC_{50}) in a mammalian Vero cell line till 62.5 μg/mL concentrations.[10] After 72 h of exposure, viability was assessed on the basis of cellular conversion of MTT into a formazan product and the results are reported in Table 1. Eighteen compounds when tested showed IC_{50} values ranging from 139.1 to >215.2 μM. These results are important as these compounds with their increased cytoliability, are much less attractive in the development of a compounds for the treatment of TB. This is primarily due to the fact that the eradication of TB requires a lengthy course of treatment, and the need for an agent with a high margin of safety becomes a primary concern. The IC_{50} values of compound **4r** was found to be 139.1 μM and showed selectivity index (IC_{50}/MIC) of 632.2.

Screening of the antimycobacterial activity of these 5-nitrofuran-2-yl derivatives, identified *N*-(3,5-dibromopyridin-2-yl)-2-((5-nitrofuran-2-yl)methylene)hydrazinecarbothioamide (**4r**) as a new lead endowed with high activity towards log-phase and starved MTB, and NTM. The present study reveals the importance of these compounds effective for the treatment of TB, and NTM infections. In conclusion, it has been shown that the potency, selectivity, and low cytotoxicity of these compounds make them valid leads for synthesizing new compounds that possess better activity with low cytotoxicity. Further structure–activity and mechanistic studies should prove fruitful.

Acknowledgments

The authors are thankful to Department of Biotechnology (BT/01/COE/05/06/01), Government of India for their financial assistances.

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