Bioorganic & Medicinal Chemistry Letters 22 (2012) 7414–7417

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Development of 5-nitrothiazole derivatives: Identification of leads against both replicative and latent *Mycobacterium tuberculosis*

Variam Ullas Jeankumar [a], Manoj Chandran [a], Ganesh Samala [a], Mallika Alvala [a], Pulla Venkat Koushik [a], Perumal Yogeeswari [a], Elena G. Salina [b], Dharmarajan Sriram [a,*]

[a] *Antitubercular Drug Discovery Laboratory, Department of Pharmacy, Birla Institute of Technology& Science - Pilani, Hyderabad Campus, Jawahar Nagar, Hyderabad 500 078, India*
[b] *Institution of the Russian Academy of Sciences A.N. Bach Institute of Biochemistry RAS, Moscow, Russia*

ARTICLE INFO

Article history:
Received 3 July 2012
Revised 25 September 2012
Accepted 13 October 2012
Available online 23 October 2012

Keywords:
Antitubercular activity
Mycobacterium tuberculosis
Nitrothiazole derivatives
Dormant tuberculosis

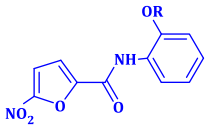
ABSTRACT

Twenty eight 5-nitrothiazole derivatives were synthesized and evaluated for in vitro activities against *Mycobacterium tuberculosis* (MTB), cytotoxicity against HEK 293T. Among the compounds, 5-nitro-*N*-(5-nitrothiazol-2-yl)furan-2-carboxamide (**20**) was found to be the most active compound in vitro with MICs of 5.48 μM against log-phase culture of MTB and also non-toxic up to 100 μM.

Since 1993, when the World Health Organization (WHO) declared tuberculosis (TB) a global emergency, there has been tremendous progress in the fight against the disease. Directly observed therapy short-course (DOTS), the recognized case management approach for TB, has been implemented in the 184 countries that account for 99 percent of all estimated TB cases. The global burden of TB is falling slowly, and at least three regions in the world are on track to achieve global targets for halving the number of cases and deaths by 2015.[1] Although TB is curable and preventable, one of three people in the world is currently latently infected with the TB bacterium without overt symptoms of disease, and the active form of the disease kills 1.8 million people annually. Co-infection with TB and HIV(TB/HIV) and a surge in multidrug-resistant TB (MDR–TB) and extensively drug-resistant TB (XDR–TB) are threatening to disrupt recent global successes in TB control. The drugs currently used to treat TB were discovered more than 40 years ago and do not meet the demands of today's TB epidemic. Specific and high effective anti-latent TB drugs are still not found and traditional antibiotics such as isoniazid and rifampin are currently being used for latent TB curing but the effectiveness of such treatment of latent infection is rather controversial. [2,3]

TB requires treatment with multiple drugs, and existing regimens require at least six months of monitored use to cure. They are burdensome for patients and care providers alike, which can lead to poor adherence among patients. Those who do not or cannot complete their treatment may develop drug-resistant TB strains that take up to two years to treat with second-line drugs, often with severe side effects.[4] There is an urgent need for novel TB drug regimens that cure more rapidly, and for drugs that can be safely taken concurrently with antiretroviral therapies.[5] Recently Nitazoxanide (NTZ) and its deacetylated active metabolite Tizaxonide (TIZ) were reported with its potency against killing replicative and non-replicative *Mycobacterium tuberculosis* (MTB) by a novel mechanism of action (Fig. 1).[6] NTZ, a 5-nitro-thiazolyl anti-parasitic drug kills MTB by disruption of MTB's membrane potential and pH homeostasis.[7] In this paper we report the antitubercular activity of various 5-nitrothiazolyl derivatives; which are structural analogues of NTZ.

The general synthetic pathway followed to achieve the title compounds is outlined in Figure 2 (Scheme 1 and 2). In the present protocol (Scheme 1) the hydroxyl (–OH) group of various

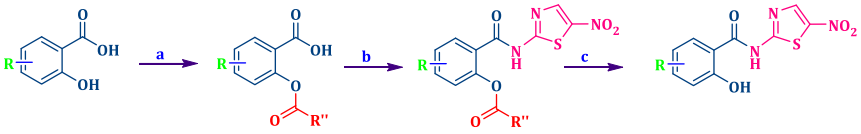


	R	MIC
NTZ	-$COCH_3$	16 μg/mL
TIZ	-H	16 μg/mL

Figure 1. Structure of NTZ and TIZ.

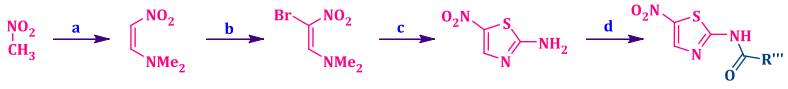
* Corresponding author.
E-mail address: dsriram@bits-hyderabad.ac.in (D. Sriram).

0960-894X/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2012.10.060



a. R''COCl, TEA, DCM; b. i. $(COCl)_2$/DMF, DCM, ii. 2-Amino-5-nitrothiazole, DIPEA, DCM; c.6N HCl, reflux

Scheme 1

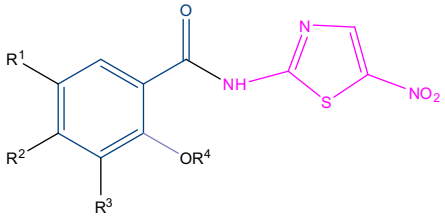


a. NaOAc; b. Br_2; c. Thiourea; d. R'''COCl, DIPEA, DCM

Scheme 2

Figure 2. General synthetic procedure.

Table 1
Physical constants and biological data of compounds **1–18**



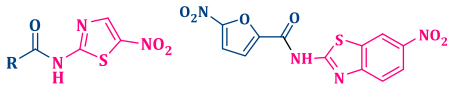
Compound	R^1	R^2	R^3	R^4	Yield in %	MP in °C	MTB MIC in μM[a]	% Growth 25 μm[b]	% Growth 100 μm[b]
1	H	Cl–	H	–OH	69	163	>166.83	ND	ND
2	H	Cl–	H	$-COCH_3$	55	157	36.57	100	83.46
3	H	Cl–	H	$-COC_6H_5$	60	173	>123.82	ND	ND
4	Cl–	H	H	–OH	65	178	20.85	100	63.03
5	Cl–	H	H	$-COCH_3$	55	169	18.28	59.49	31.15
6	Cl–	H	H	$-COC_6H_5$	73	171	15.47	72.16	23.7
7	Cl–	H	Cl–	–OH	65	177	74.81	100	23.47
8	Cl–	H	Cl–	$-COCH_3$	58	175	66.45	100	95.1
9	Cl–	H	Cl–	$-COC_6H_5$	55	160	28.52	100	61.59
10	CH_3O–	H	H	–OH	67	191	84.66	100	100
11	CH_3O–	H	H	$-COCH_3$	72	158	74.11	100	94.14
12	CH_3O–	H	H	$-COC_6H_5$	75	165	15.64	100	67.57
13	NO_2–	H	NO_2–	–OH	66	164	>140.74	ND	ND
14	NO_2–	H	NO_2–	$-COCH_3$	70	159	>125.85	ND	ND
15	NO_2–	H	NO_2–	$-COC_6H_5$	60	157	>108.84	ND	ND
16	$(CH_3)_2CH$–	H	$(CH_3)_2CH$–	–OH	65	186	17.88	29.36	7.1
17	$(CH_3)_2CH$–	H	$(CH_3)_2CH$–	$-COCH_3$	71	201	63.86	45.93	20.56
18	$(CH_3)_2CH$–	H	$(CH_3)_2CH$–	$-COC_6H_5$	66	179	>110.25	ND	ND
NTZ	H	H	H	$-COCH_3$	—	—	162.71	ND	ND
INH	—	—	—	—	—	—	0.72	ND	ND
Rif	—	—	—	—	—	—	0.48	ND	ND

[a] Minimum inhibitory concentration against *M. tuberculosis*.
[b] Cytotoxicity against HEK 293T.

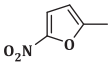
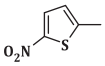
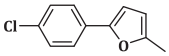
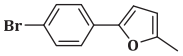
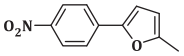
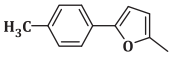
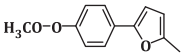
commercially available substituted salicylic acids were first subjected to acetylation/benzoylation to get the desired acetyl or benzoyl derivative of the corresponding salicylic acid in quantitative yield using well known procedures. The thus obtained product was then coupled with 2-amino-5-nitrothiazole, synthesised in quantitative yield from nitro methane via the bromination of *N,N*-dimethyl-2-nitroetheneamine and subsequent treatment with thiourea (as shown in scheme 2) by using a simple and efficient protocol as previously described by Dah-Chieh[8] to get the first set of compounds in the library. Considering the low solubility and low nucleophilicity of 2-amino-5-nitrothiazole, the coupling was achieved by first converting the corresponding substituted salicylic acid into the respective acid chloride and then coupling with amine in THF or DMF as the solvent. The second set in the library was achieved by either deacetylating or debenzoylating the above obtained derivative using 6 N HCl in THF.[9–11] Encouraged by the promising anti-tubercular activity of the above synthesised derivatives further work was initiated in our lab with the goal of obtaining a lead series with tractable SAR and potencies better than the above compounds and thus the second series (Scheme 2) in the library was designed and synthesised as shown in Table 1 and 2. Both analytical and spectral data (^{1}H NMR, ^{13}C NMR, and mass spectra) of all the synthesized compounds were in full agreement with the proposed structures.[12]

All the new 5-nitrothiazole derivatives were screened for their *in-vitro* anti-tubercular activity against *Mycobacterium tuberculosis*

Table 2
Physical constants and biological data of compounds **19–28**



19-27 **28**

Compound	R	Yield in %	MP in °C	MTB MIC in μM[a]	% Growth 25 μm[b]	% Growth at 100 μm[b]
19		65	177	205.55	ND	ND
20		80	185	5.48	87.44	67.26
21		72	183	24.48	100	93.44
22		77	202	20.81	100	35.94
23		64	179	17.86	75	74.12
24		63	206	31.7	89.14	70.34
25		71	239	17.34	88.44	79.22
26		69	211	18.97	94.1	91.54
27		67	187	72.39	100	95.66
28	—	70	206	149.58	100	43.33
NTZ	—	—	—	162.71	ND	ND
INH	—	—	—	0.72	ND	ND
Rif	—	—	—	0.48	ND	ND

[a] Minimum inhibitory concentration against *M. tuberculosis*.
[b] Cytotoxicity against HEK 293T.

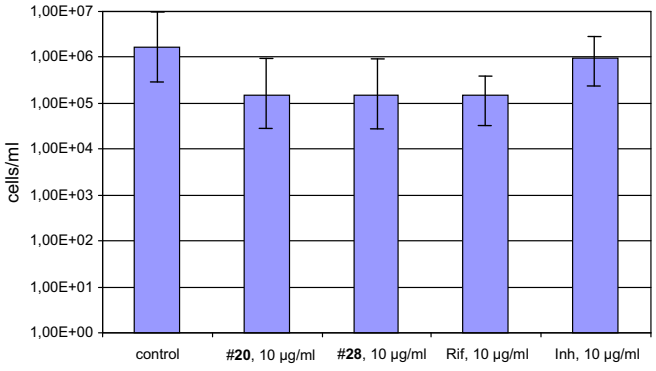


Figure 3. The effectiveness of the compounds **20** and **28** for killing *M. tuberculosis* 'non-culturable' cells. 'Non-culturable' cells were washed and treated by 10 μg/ml of rifampicin (Rif), isoniazid (Inh) and compounds #20 and 28 for 7 days. Viability of both treated and untreated 'non-culturable' cells was tested by the concentration of cells which were able to recover from NC state by MPN assay.

H37Rv (ATCC27294) using an agar dilution method using drug concentration from 50 μg/mL to 0.78 μg/mL in duplicates. The minimum inhibitory concentration (MIC) was determined for each compound. The MIC is defined as the minimum concentration of compound required to completely inhibit the bacterial growth. NTZ, Isoniazid and Rifampicin were used as a reference compound and its MIC was found to be 50 μg/mL in our screening. The MIC values of the synthesized compounds along with the standard drug for comparison were reported in Table 1 and 2. Among the twenty eight compounds screened twenty two compounds showed activity against MTB with MIC ⩽50 μg/mL. Eleven compounds (**4–6, 12, 16, 20–23, 25–26**) inhibited MTB with MIC of less than 25 μM. Twenty one compounds were found to be more potent than standard drug NTZ. Compound **20** (5-nitro-*N*-(5-nitrothiazol-2-yl)-furan-2-carboxamide) was found to be the most active compound in vitro with MICs of 5.48 μM against log-phase culture of MTB and was 29 times more potent than NTZ.

With respect to structure-TB activity relationship, we prepared eighteen salicylanilides derivatives (**1–18**) and ten hetero-aryl amide derivatives (**19–27**). Among salicylanilides derivatives, we have done modification at *o* position of phenyl ring with hydroxyl, acetoyl and benzoyl derivatives (R_4), *m*-position with chloro, nitro, isopropyl and hydrogen (R_3), *p*-position with chloro and hydrogen and 5th position with hydrogen (R_2), chloro, methoxyl, nitro and isopropyl groups (R_1). Among *p*-chloro and 5-chloro derivatives (**1–3** vs **4–6**) 5-chloro derivatives were found to be more active, and they are also showed more promising than 3,5-dicholoro derivatives (**7–9**). Methoxyl group at 5th position and isopropyl group at 3rd and 5th position also retain activity (**10–12, 16–18**). Introduction of nitro groups at 3rd and 5th position is detrimental for activity (**13–15**). When compared to NTZ with MIC of 162.71 μM; introduction of *p*-chloro group (**2**) enhances 4.4 times more active, 5-chloro group (**5**) enhances 8.9 times, 3,5-dichloro groups (**8**) enhances 2.44 times, 5-methoxyl group (**11**) enhances 2.19 times, and 3,5-diisopryl groups (**17**) enhances 2.54 times more activity. Whereas introduction of 3,5-dinitro groups (**14**) makes the NTZ inactive. Among the substituent's at *o*-position, order of activity is benzoyl>acetoyl>hydroxyl, in 5-chloro, 5-methoxyl and 3,5-diisopropyl derivatives. Among hetero-aryl amides (**19–27**), 2-thienyl derivative (**21**) was found to be more potent than 2-furanyl derivative (**19**). Introduction of 5-nitro group at 2-furanyl ring (**20**) enhances the activity 37.5 times, whereas

introduction of 5-nitro group at 2-thienyl group not showed any appreciable activity. Introduction of phenyl ring at 5th position of 2-furanyl ring enhances the activity in many folds (MIC of 17.34–72.39 μM), substituents in phenyl ring; presence of *para* chloro, bromo, nitro and methyl group are beneficial for activity. Compound **20** (5-nitro-*N*-(5-nitrothiazol-2-yl)furan-2-carboxamide) was found to be most promising molecule with MICs of 5.48 μM and replacement of its 5-nitrothiazole moiety with 6-nitrobenzthiazole leads to 5-nitro-*N*-(6-nitrobenzo[d]thiazol-2-yl)furan-2-carboxamide (**28**). Compound **28** (MIC of 149.58 μM) was 27.2 times less potent than **20**, but slightly more potent than NTZ.

Compounds **20** and **28** were also examined for their activity against dormant MTB bacilli at 10 μg/mL. To obtain dormant cells, *M. tuberculosis* bacilli were grown in potassium-deficient Sauton's medium supplemented with ADC and 0.05% of Tween-80 (37 °C, 200 rpm). 'Dormancy' was detected by inability of the cells to form colonies onto agar solidified Sauton's medium. Resuscitation of both treated and untreated NC cells was performed in liquid Sauton's medium, with the concentration of cells recovered from dormancy being estimated by Most Probable Numbers (MPN) assay and with the use of statistical approaches.[13] It was found that after treatment with compounds dormant cells were less able to recover from dormancy (Fig. 3). Both compounds caused a ~1-log decrease in the viability of dormant cells after incubation with 10 μg/ml for 7 days. Although this effect is quite a modest one the activity of these compounds exceeds the effectiveness of isoniazid and is comparable to rifampicin in relation to dormant *M. tuberculosis* bacilli. These compounds may be regarded as the prominent compound for the development of derivatives which are more effective for dormant MTB cells and latent tuberculosis.

Compounds which showed reasonable anti-TB activity were also tested for in-vitro cytotoxicity against HEK293Tcells at 25 and 100 μM concentrations by (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Percentage growth of cells was reported in Table 1,2. The most promising anti-TB compound **20** showed 32% inhibition at 100 μM with therapeutic index of >20. Compound **16** was found to be cytotoxic in 25 μM itself.

In this work we identified some novel 5-nitrothiazolyl derivatives with activity against both replicative and dormant MTB.

Acknowledgments

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

References and notes

1. World Health Organization, 2011/2012 Tuberculosis Global Facts, Geneva, Switzerland (**2011**).
2. Koul, A.; Arnoult, E.; Lounis, N.; Guillemont, J.; Andries, K. *Nature* **2011**, *469*, 483.
3. Chao, M. C.; Rubin, E. J. *Annu. Rev. Microbiol.* **2010**, *64*, 293.
4. Stewart, T. C.; Giovanna, R. *Curr. Opin. Microbiol.* **2011**, *14*, 570.
5. Daniela, J. *Curr. Opin. Microbiol.* **2011**, *14*, 564.
6. LuizPedro, S. D.; Gang, L.; Xiuju, J.; Carl, N. *J. Med. Chem.* **2009**, *52*, 5789.
7. Luiz Pedro, S. D.; Crystal, M. D.; Kyu, Y. R.; Carl, N. *ACS. Med. Chem. Lett.* **2011**, *2*, 849.
8. Dah-Chieh, O.C.; Kim, S.C. US Patent 4,269,985.
9. Rossignol, J. F.; Cavier, R. *Chem. Abstr.* **1975**, *83*, 28216.
10. Eric, B. T.; Wang, X.; Igor, O.; Taylor, K.; Craig, S.; Joseph, S.; Michelle, W.; Paul, S. H.; Timothy, L. M. *Chem. Med. Chem.* **2011**, *6*, 362.
11. Andrew, V. S.; Chandrakala, P.; Eleanor, R.; Raman, S.; Neil, G. B.; Mazhar, I.; Joanne, B.; Sarah, A. A.; Geoffrey, E.; Alison, H.; Jennifer, H.; Brent, K.; Edward, J. S.; Jean, F. R. *J. Med. Chem.* **2011**, *54*, 4119.
12. (a) 5-Nitro-*N*-(5-nitrothiazol-2-yl)furan-2-carboxamide (**20**): yellowish brown solid; yield (80%). ^{1}H NMR (300 MHz, DMSO-d_6) δ_H7.81 (d, J = 3.8, 1H, ArH), 7.88 (d, J = 3.8, 1H, ArH), 8.69 (s, 1H, -thiazole). ^{13}CNMR (75MHz, $CDCl_3$): δ_c 162.3, 156, 152.5, 145.3, 141.6, 141.3, 119.1, 113.1. Anal. Calcd for $C_8H_4N_4O_6S$:C33.81; H, 1.42; N, 19.71. Found: C, 33.88, H, 1.39; N, 19.68. MS *m/z:* 283.94 [M$^+$].
(b) 4-Chloro-2-(5-nitrothiazol-2-ylcarbamoyl)phenyl benzoate (**6**): yellow solid; yield (73%).^{1}H NMR (300 MHz, DMSO-d_6) δ_H7.67–8.16 (m,8H, ArH), 8.63 (s, 1H, -thiazole). ^{13}CNMR (75MHz, $CDCl_3$): δ_c 166.4, 162.8, 144.8, 143.2, 136.5, 134.1, 133.8, 131.4, 129.8, 128.1, 127.3, 126.4, 122.6. Anal. Calcd for $C_{17}H_{10}ClN_3O_5S$:C, 50.57; H, 2.50; N, 10.41. Found: C, 50.53; H, 2.51; N, 10.39.MS *m/z:* Found [M+*m/z*:%] 403.04, 100; 405.04, 37.1.
13. deMan, J. C. *J. Appl. Microbiol.* **1975**, *1*, 67.