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# Potential Tuberculostatic Agents. Topliss Application on Benzoic Acid [(5-Nitro-thiophen-2-yl)-methylene]-hydrazide Series

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**Abstract**—Nitroaromatic compounds such as nifuroxazide are used in many human enteropathogenic bacteria infections without causing an increase in the plasmidial antibiotic resistance of the aerobic Gram-negative intestinal Enterobacteriaceae. For these reasons, these compounds have been synthesized using the rational approach of Topliss' decision tree. Generally, this approach allows us to obtain the most active derivative from the series in a few steps. These compounds were tested against *Mycobacterium tuberculosis* in vitro and the most active of the series identified. A new lead for potential tuberculostatic activity has been predicted and will be used in further QSAR studies. © 2002 Elsevier Science Ltd. All rights reserved.

## Introduction

Tuberculosis remains a major public health issue at the beginning of the 21st century. Only in the last decade, TB caused nearly 3 million deaths annually. The estimated 8.8 million new cases every year correspond to 52,000 deaths per week or more than 7000 each day. In developing countries tuberculosis is a leading cause of morbidity and mortality (Fig. 1). War, famine, homelessness and the spread of the HIV epidemic has significantly contributed to the worsening of the situation.[1]

Co-infection with HIV has been responsible for the changes in the TB epidemiologic situation and also for the emergence of multidrug-resistant strains.[2]

Because of this critical situation, much effort has been directed to the design of new drugs for tuberculosis therapeutics.

Nitroaromatic compounds, such as nifuroxazide, have been used in many human enteropathogenic bacterial infections without causing an increase in the plasmidial antibiotic resistance of the aerobic Gram-negative intestinal Enterobacteriaceae. It is known that reduction of the nitro group is essential for the biological activity of these compounds and this may be responsible for the production of free radicals which could act on DNA.[3]

Nitrothiophenes have already been tested against parasitic infections and have demonstrated good results against *Entamoeba histolytica*, *Trichomonas vagi*nalis, *Leishmania tropica* and *L. infantum*. Structure–activity studies suggested that an increase in conjugation on the thiophenic moiety leads to an increase in biological activity.[4]

Considering the urgent need for chemotherapeutical alternatives against tuberculosis, particularly after the emergence of multidrug-resistant strains, and in view of the high activity shown by nifuroxazide, as already mentioned, the objective of this work was to obtain the most active derivative from the benzoic acid 5-nitro-thiophen-2-yl)-methylene]-hydrazides series, nifuroxazide analogues. For this purpose we have applied Topliss' methodology[5] to a set of nitrothiophen analogues (Fig. 2). All compounds have been synthesized and their activity evaluated against *Mycobacterium tuberculosis* Rv strain.

The fundamental structure is a nitrofurane analog, designed by classical bioisosterism between the oxygen and the sulfur atom ring.

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## Results

The set of substituents were selected in accordance with Topliss' methodology as shown in Figure 3.

Simultaneously, $\log P_{app}$ values for this set of compounds were determined by HPLC method. The physicochemical and biological results used in the analysis are depicted in Table 1.

## Discussion

The compounds were easily synthesized by trivial methods, with good yields (95%) and their structures were confirmed by spectrometric analysis.

The biological parameter, expressed by MIC values for all compounds of the set, was evaluated by a microdilution test against the *M. tuberculosis* Rv strain (Table 1). All compounds but 4-Cl substituted derivative showed activity. This is much promising, considering the complexity of mycobacteria wall. Compound **3** (R = 4-OMe) was the most active being almost seven times more potent than the unsubstituted derivative (R = H), compound **1**, demonstrating MIC values of 6.6 and 45.6 nmol/mL, respectively.

This is a promising compound, considering that isoniazid, the first drug of choice for tuberculosis treatment, exhibits a MIC value of 3.6 nmol/mL.

Considering Figure 2, the nature of the physicochemical properties of the substituent of the most active compound, according to the Topliss approach, indicates, in general, that more potent compounds should be obtained with derivatives having substituents that show both electron donor ($\sigma < 0$) and hydrophilic character.

With the objective of verifying the contribution of the electronic parameter, the derivative 4-$N(CH_3)_2$ substituted ($\sigma = -0.83$; $\log P_{app} = 2.54$) was synthesized. This compound showed a $\log P_{app}$ close to that of compound **3** ($\sigma = -\log P_{app} = 2.58$). Considering that $\log P_{app}$ includes ionization correction, the inclusion of unionizable disubstituted compound (3-$CH_3$, 4-$OCH_3$), suggested by Topliss' decision tree, was not necessary. Although it seems that in vitro inhibition of *M. tuberculosis* Rv strain is mainly described by the electronic parameter, we could not assert this, since in this set the two other substituents—OH ($\sigma = -0.37$; $\log P_{app} = 2.04$); $NH_2$ ($\sigma = -0.66$; $\log P_{app} = 1.49$)—showed both electronic and hydrophobic parameters varying almost in a parallel manner.

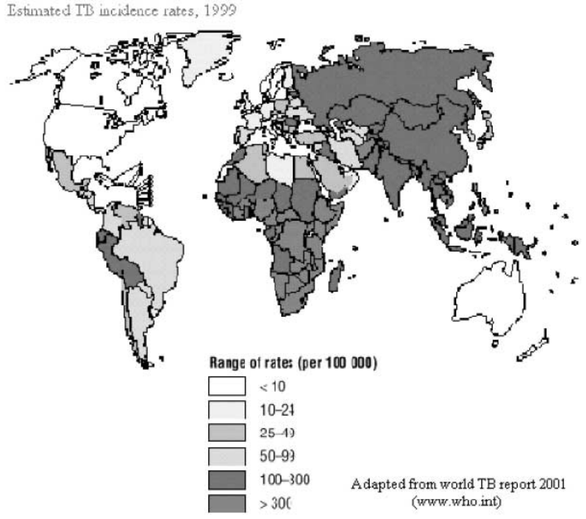


**Figure 1.** Tuberculosis world epidemiological situation.[1]

In conclusion, by applying the Topliss approach to the benzoic acid-[(5-nitro-thiophen-2-yl)-methylene]-hydrazide set, a new lead for potential tuberculostatic activity has been predicted. Although we have promising results applying this manual method, as mentioned above, we know its limitation concerning mainly a not necessarily spanned substituent space, leading to a colinearity among the physico-chemical variables, and flexibility. However, this is a starting point to further QSAR studies.

## Experimental

### Synthesis of benzoic acid [(5-nitro-thiophen-2-yl)-methylene]-hydrazides substituted derivatives: general method

The analogues were synthesized from benzoic acids substituted with hydrazine and 5-nitrothiophyilidene methanediol diacetate.

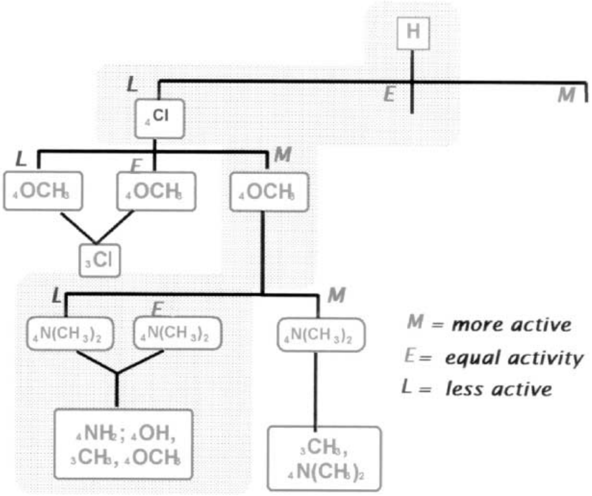
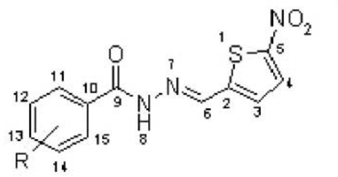


**Figure 2.** Topliss' branch followed in this set.



| R | Compound |
|---|---|
| 4-H | 1 |
| 4-Cl | 2 |
| 4-OMe | 3 |
| 4-$N(CH_3)_2$ | 4 |
| 4-$NH_2$ | 5 |
| 4-OH | 6 |

**Figure 3.** Set of 5-nitro-2-thiophylidene derivatives synthesized.

**Table 1.** Biological and physical chemical data of substituted benzoic acid-[(5-nitro-thiophen-2-yl)-methylene]-hydrazides

| Compound | MIC[a] (μg/mL) | MIC (nmol/L) | ClogP[b] | $\log P_{app}$[c] |
|---|---|---|---|---|
| **1** | 12.5 | 45.6 | 2.07 | 2.41±0.01 |
| **2** | >200 | >646.2 | 2.98 | 3.27±0.01 |
| **3** | 2.0 | 6.6 | 2.27 | 2.58±0.01 |
| **4** | 4.0 | 12.6 | 2.67 | 2.54±0.01 |
| **5** | 4.0 | 13.8 | 1.62 | 1.49±0.01 |
| **6** | 4.0 | 13.7 | 2.09 | 2.04±0.01 |
| Isoniazid | 0.5 | 3.6 | −0.74 | −0.70[d] |

[a] Obtained by microdilution methodology.
[b] Obtained by ClogP for Windows version 1.0.0, 1995, BioByte Corp., USA.
[c] Values obtained at pH 7.40 (Trizma buffer, μ = 0.10 M, NaCl); t = 23 ± 1 °C.
[d] Taken from literature.[6]

The methyl esters were obtained from reflux of the respective substituted benzoic acids (1 mmol) and methanol (≈10 mmol) in the presence of $H_2SO_4$. Resultant crystals were filtrated, washed with distilled water and dried in a desiccator under phosphorus pentoxide.

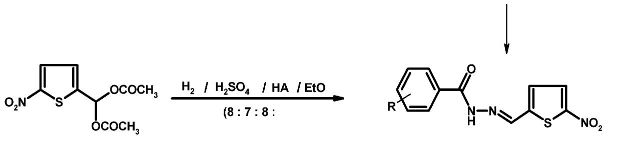
The respective benzhydrazides were synthesized from these methyl esters (1 mmol) and hydrazine 35% (30 mmol) under reflux for 1 h. These derivatives were isolated by the same method used for the esters.

Target compounds were synthesized by the formation of imines from equimolar proportion of substituted benzhydrazides and 5-nitrothiofene methanediol diacetate in ethanol/acetic acid/sulfuric acid/water (20:8:7:8). They were filtrated and recrystallized from *N,N*-dimethylformamide (DMF).[7,8]

Scheme 1 summarizes these reactions.

The structural analysis of these compounds is discussed as follows where mp = melting point and * indicates elemental analysis calculated corresponds to a water molecule adsorbed.

**Benzoic acid [(5-nitro-thiophen-2-yl)methylene]-hydrazide.** The obtained compound is a dark-yellow amorphous solid. Yield: 96.35%; mp 220–223 °C. $^1$H NMR: δ 7.56–7.69 (m, 3H, $H_{12}$, $H_{13}$, $H_{14}$), 7.95 (d, 2H, $H_{11}$, $H_{15}$), 8.17 (d, 1H, $H_6$), 8.70 (d, 1H, $H_4$), 9.04 (d, 1H, $H_3$), 12.25 (s, 1H, $H_8$); $^{13}$C NMR: δ 128.60 ($C_{12}$ ad $C_{14}$), 129.44 ($C_{11}$ and $C_{15}$), 130.51 ($C_{13}$ and $C_{10}$), 131.38 ($C_3$ and $C_4$), 142.50 ($C_6$), 147.60 ($C_2$), 164.00 ($C_9$); EA*: calcd C, 52.36%; H, 3.30%; N, 15.26%. Experimental: C, 50.99%; H, 3.33%; N, 15.34%.



**Scheme 1.** General synthesis of benzoic acid [(5-nitro thiophen-2-yl)-methylene]-hydrazides.

**4-Chloro-benzoic acid [(5-nitro-thiophen-2-yl)methylene]-hydrazide.** The obtained compound is a light-yellow amorphous solid. Yield: 71.23%; mp 272–275 °C. $^1$H NMR: δ 7.77–7.80 (m, 3H, $H_4$, $H_{12}$ $H_{14}$), 8.10 (d, 2H, $H_{11}$ and $H_{15}$), 8.32 (d, 1H, $H_3$), 8.84 (s, 1H, $H_6$), 12.50 (s, 1H, $H_8$); $^{13}$C NMR: δ 128.61 ($C_4$), 129.65 ($C_{12}$ and $C_{14}$), 129.85 ($C_{11}$ and $C_{15}$), 130.50 ($C_3$), 131.52 ($C_{10}$), 137.01 ($C_2$), 141.48 ($C_{13}$), 146.50 ($C_6$), 147.00 ($C_5$), 162.20 ($C_9$); EA: calcd C, 43.98%; H, 3.08%; N, 13.76%. Experimental: C, 44.36%; H, 3.18%; N, 12.54%.

**4-Methoxy-benzoic acid [(5-nitro-thiophen-2-yl)-methylene]-hydrazide.** The obtained compound is a dark-yellow amorphous solid. Yield: 81.51%; mp 195 °C (decomposition). $^1$H NMR δ 3.84 (s, 3H, $H_{17}$), 7.07 (d, 2H, $H_{12}$ and $H_{14}$), 7.55 (d, 1H, $H_4$), 7.89 (d, 2H, $H_{11}$ and $H_{15}$), 8.12 (d, 1H, $H_3$), 8.66 (sl, 1H, $H_6$), 8.94 (S, 1H, $H_8$); $^{13}$C NMR: δ 55.40 ($C_{17}$), 113.73 ($C_{12}$ and $C_{14}$), 124.74 ($C_{10}$), 129.28 ($C_4$), 129.68 ($C_{11}$ and $C_{15}$), 130.45 ($C_3$), 140.42 ($C_2$), 146.91 ($C_6$), 150.56 ($C_5$), 162.23 ($C_{13}$), 162.67 ($C_9$); EA*: calcd C, 51.14%; H, 3.63%; N, 13.76%. Experimental: C, 50.23%; H, 3.54%; N, 14.00%.

**4-Dimethylamine-benzoic acid [(5-nitro-thiophen-2-yl)-methylene]-hydrazide.** The obtained compound is an orange amorphous solid. Yield: 92.45%; mp 255–257 °C. $^1$H NMR: δ 3.00 (s, 6H, $H_{17}$ and $H_{17'}$), 6.76 (d, 2H, $H_{12}$ and $H_{14}$), 7.53 (d, 1H, $H_4$), 7.81 (d, 2H, $H_{11}$ and $H_{15}$), 8.13 (d, 1H, $H_3$), 8.66 (s, 1H, $H_6$), 11.85 (s, 1H, $H_8$); $^{13}$C NMR: δ 40.26 ($C_{17}$ and $C_{17'}$), 110.53 ($C_{12}$ and $C_{14}$), 118.58 ($C_{10}$), 128.87 ($C_4$), 129.38 ($C_{11}$ and $C_{15}$), 130.56 ($C_3$), 139.35 ($C_2$), 140.90 ($C_6$), 147.43 ($C_{13}$), 150.32 ($C_5$), 154.33 ($C_9$); EA: calcd C, 52.83%; H, 4.40%; N, 17.61%. Experimental: C, 50.92%; H, 4.27%; N, 17.30%.

**4-Amino-benzoic acid [(5-nitro-thiophen-2-yl)methylene]-hydrazide.** The obtained compound is an orange amorphous solid. Yield: 95.39%; mp 260 °C (decomposition). $^1$H NMR: δ 5.87 (s, 2H, $H_{16}$), 6.60 (d, 2H, $H_{12}$ and $H_{14}$), 7.51 (d, 1H, $H_4$), 7.66 (d, 2H, $H_{11}$ and $H_{15}$), 8.12 (s, 1H, $H_3$), 8.63 (s, 1H, $H_6$), 11.83 (s, 1H, $H_8$); $^{13}$C NMR: δ 112.49 ($C_{12}$ and $C_{14}$), 118.59 ($C_{10}$), 128.67 ($C_{11}$ and $C_{15}$), 129.59 ($C_4$), 130.48 ($C_3$), 138.90 ($C_2$), 147.44 ($C_6$), 150.22 ($C_5$), 152.62 ($C_{13}$), 163.20 ($C_9$). EA*: calcd C, 52.83%; H, 4.40%; N, 17.61%. Experimental: C, 50.92%; H, 4.27%; N, 17.30%.

**4-Hydroxi-benzoic acid [(5-nitro-thiophen-2-yl)-methylene]-hydrazide.** The obtained compound is a yellow amorphous solid. Yield: 95.09; mp 260 °C (decomposition). $^1$H NMR: δ 6.87 (d, 2H, $H_{12}$ and $H_{14}$), 7.55 (d, 1H, $H_3$), 7.80 (d, 2H, $H_{11}$ and $H_{15}$), 8.12 (d, 1H, $H_4$), 8.66 (s, 1H, $H_6$), 12.02 (s, 1H, $H_8$); $^{13}$C NMR: δ 115.02 ($C_{12}$ and $C_{14}$), 123.13 ($C_4$), 129.13 ($C_{11}$ and $C_{15}$), 129.85 ($C_{10}$), 130.45 ($C_3$), 140.02 ($C_2$), 147.02 ($C_6$), 150.48 ($C_5$), 160.95 ($C_{13}$), 161.01 ($C_9$). EA: calcd C, 49.48%; H, 3.11%; N, 14.43%. Experimental: C, 48.76%; H, 3.09%; N, 14.32%.

Melting points were determined using Büchi CHI apparatus. Elemental composition was determined by a Perkin–Elmer 24013-CHN Elemental Analyser. NMR spectra were recorded with a Bruker $DPX_{300}$ (300 MHz) spectrometer on DMSO-$d_6$ solutions with tetramethylsilane as internal standard.

$\log P_{app}$ values of synthesized compounds were determined by means of a reversed-phase HPLC capacity factor procedure,[9] using a 1-octanol coated Superguard LC-18-DB column (mean particle size 4 mm, and 2.0 cm length), as the stationary phase. The mobile phase was 1-octanol saturated Trizma hydrochloride buffer [50.0 mM Tris (hydroxymethyl)aminomethane hydrochloride) adjusted to pH 7.40 with 0.10 M HCl] and to an ionic strength of 0.10 M (NaCl). All the measurements were performed at room temperature controlled to 23 ± 1 °C. The $\log P_{app}$ values were calculated from HPLC capacity factors ($\log k'$).

Minimal inhibitory concentrations (MIC) were determined using microdilution plates and Alamar Blue as vital dye according to the methodology of Franzblau et al.[10] The bacterial suspension used was prepared by adjusting its turbidity by the MacFarland no. 1 scale, and further dilution (1:25) in culture medium $7H_9$. Twofold serial dilution of the compounds was used to titrate the plates. The concentrations ranged from 0.25 to 200 µg/mL, using isoniazid as drug control. The maintenance of a blue color in the wells is considered to demonstrate lack of bacterial growth and the development of a pink color is interpreted as the converse. MIC is, thus, considered as the minimum concentration able to inhibit the change of color from blue to pink.

## Acknowledgement

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