Design, synthesis and antimycobacterial evaluation of some new azaheterocycles with 4,7-phenanthroline skeleton. Part VI

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Abstract: A feasible study concerning the synthesis, structure and *in vitro* antimycobacterial evaluation of new 4,7-phenanthroline derivatives is reported. The preparation is straight and efficient, involving an *N*-alkylation reaction of 4,7-phenanthroline. The structure of the new compounds have been proved by elemental and spectral (IR, ^{1}H and ^{13}C NMR) analysis. The *in vitro* antimycobacterial evaluation of five synthesized compounds was investigated against *Mycobacterium tuberculosis H37Rv* under aerobic conditions, two of them being active. A certain influence of substituents from the *para* position of the benzoyl moiety was observed, the 4,7-phenanthrolin-4-ium salt substituted with (*p*)chloro-benzoyl showing the most pronounced antimycobacterial activity.

Keywords: (*p*)Halogeno-benzoyl, cycloimmonium salts.

RUNNING TITLE: SYNTHESIS OF NEW 4,7-PHENANTHROLINE DERIVATIVES

INTRODUCTION

Phenanthroline derivatives attracted attention especially due to their biological effects,[1-6] crystal engineering,[7-11] their unique π-electrons delocalization[12,13] and complexation properties especially in the case of 1,10-phenanthroline.[14,15] While 1,10-phenathroline derivatives have been widely studied both for synthesis and applications, much less interest has been shown for the other phenanthrolines because of difficulties in their synthesis.

However, there are several reports regarding biological properties of 4,7-phenanthroline and its derivatives as inhibition of several enzymes,[16-18] bactericide activity especially as amoebicid[19] or antiviral activity.[20] More recently, 4,7-phenanthrolines derivatives were found to stabilize triple-helix DNA[21] or to possess *in vitro* and *in silico* antiviral activity against single-stranded positive-sense RNA genome viruses.[22]

As part of our ongoing research in the field of heterocyclic compounds, especially in the synthesis of (aza)indolizine and poly(aza)indolizine derivatives *via* 3+2 cycloaddition of cycloimmonium ylides,[23-25] and encouraged by our previous promising results in the field of anti-TB derivatives with nitrogen heterocycle skeleton,[26-30] we decided to study the synthesis, structure and *in vitro* antimycobacterial activity of new 4,7-phenanthrolin-4-ium salts.

EXPERIMENTAL

Chemistry

Melting points were recorded on an A. Krüss Optronic Melting Point Meter KSPI and are uncorrected. Proton and carbon nuclear magnetic resonance (δ_H, δ_C) spectra were recorded on a DRX-500 Bruker (500 MHz). All chemical shifts are quoted on the δ-scale in ppm. Coupling constants are given in Hz. IR spectra were recorded on a FTIR Shimadzu Prestige 8400s spectrophotometer. All commercially available products were used without further purification unless otherwise specified.

*General procedure for the synthesis of 4,7-phenanthrolin-4-ium salts (**1**–**8**).*

28 mmol (1 equiv.) of 4,7-Phenanthroline was dissolved in 6 mL anhydrous acetonitrile. Then 30.8 mmol (1.1 equiv.) of reactive halide was added and the resulted mixture was stirred at reflux for 24 hours. The formed precipitate was filtered and washed with acetonitrile and diethyl ether to give the desired product.

The following compounds were synthesized:

*4-(2-(4-Tolyl)-2-oxoethyl)-4,7-phenanthrolin-4-ium bromide (**1**).*

*4-(2-(4-Methoxyphenyl)-2-oxoethyl)-4,7-phenanthrolin-4-ium bromide (**2**).*

*4-(2-(4-Nitrophenyl))-2-oxoethyl)-4,7-phenanthrolin-4-ium bromide (**3**).*

*4-(2-(4-Chlorophenyl)-2-oxoethyl)-4,7-phenanthrolin-4-ium bromide (**4**).*

*4-(2-(3-Methoxyphenyl)-2-oxoethyl)-4,7-phenanthrolin-4-ium bromide (**5**).*

*4-(2-Amino-2-oxoethyl)-4,7-phenanthrolin-4-ium iodide (**6**).*

*4-(Cyanomethyl)-4,7-phenanthrolin-4-ium bromide (**7**).*

*4-(2-Methoxy-2-oxoethyl)-4,7-phenanthrolin-4-ium bromide (**8**).*

The physical and spectral data for compounds **1-8** are given in the Supplementary material to this paper.

Microbiology

Compounds were evaluated for antimycobacterial activity against *Mycobacterium tuberculosis*, as a part of the TAACF TB screening program under direction of the US National Institute of Health, the NIAID division. Antimycobacterial activities of the compounds were performed by Center of Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) at Southern Research Institute.[31-34]

The Primary Cycle High Throughput Screening (HTS). Determination of 90 % inhibitory concentration (IC_{90}), 50 % inhibitory concentration (IC_{50}) and Minimum Inhibitory Concentration (MIC)

The *MIC* of compound was determined by measuring bacterial growth after 5 days in the presence of test compounds. Compounds were prepared as 10-point two-fold serial dilutions in DMSO and diluted into 7H9-Tw-OADC medium in 96-well plates with a final DMSO concentration of 2 %. The highest concentration of compound was 200 μM where compounds were soluble in DMSO at 10 mM. For compounds with limited solubility, the highest concentration was 50X less than the stock concentration e.g. 100 μM for 5 mM DMSO stock, 20 μM for 1 mM DMSO stock. For potent compounds, assays were repeated at lower starting concentrations. Each plate included assay controls for background (medium/DMSO only, no bacterial cells), zero growth (100 μM Rifampicin) and maximum growth (DMSO only), as well as a rifampicin dose response curve. Plates were inoculated with *M. tuberculosis* and incubated for 5 days: growth was measured by OD_{590} and fluorescence (Ex 560/Em 590) using a BioTek™ Synergy 4 plate reader. Growth was calculated separately for OD_{590} and RFU. To calculate the MIC, the 10-point dose response curve was plotted as % growth and fitted to the Gompertz model using GraphPad Prism 5. The MIC was defined as the minimum concentration at which growth was completely inhibited and was calculated from the inflection point of the fitted curve

to the lower asymptote (zero growth) (Fig. 1A). In addition dose response curves were generated using the Levenberg-Marquardt algorithm and the concentrations that resulted in 50 % and 90 % inhibition of growth were determined (IC_{50} and IC_{90} respectively) (Fig. 1B).

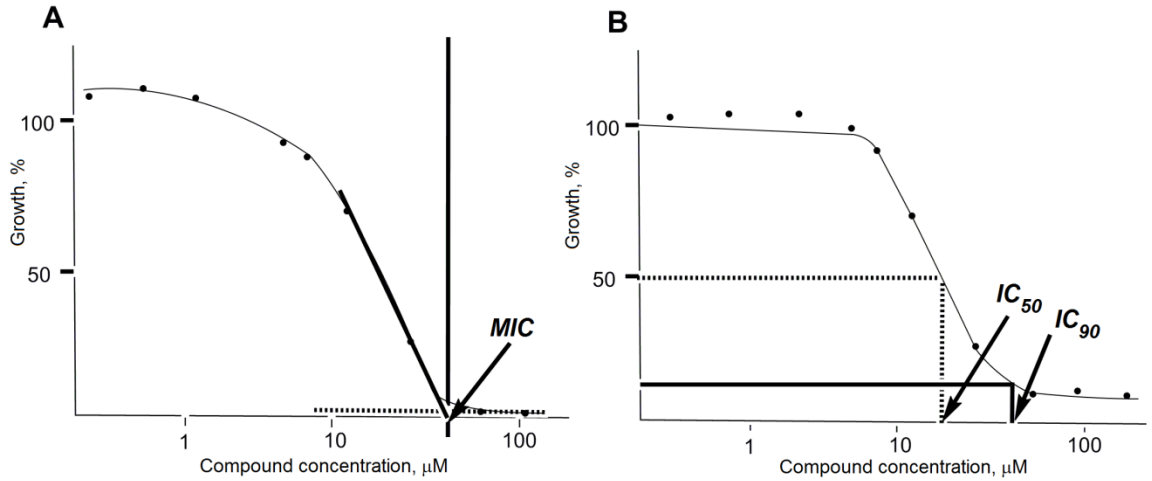


Fig. 1. Dose response curves used to calculate *MIC*, IC_{50} and IC_{90}.

Data points obtained from a dose response growth inhibition assay are curve-fitted using (A) the Gompertz model to calculate *MIC* and (B) the Levenberg-Marquardt algorithm to calculate IC_{50} and IC_{90}. (A) The *MIC* is the concentration at which complete inhibition growth is seen and is derived from the point of inflection at which the curve meets the lower asymptote (zero growth). (B) IC_{50} and IC_{90} are points at which growth is inhibited by 50 % and 90 % respectively.

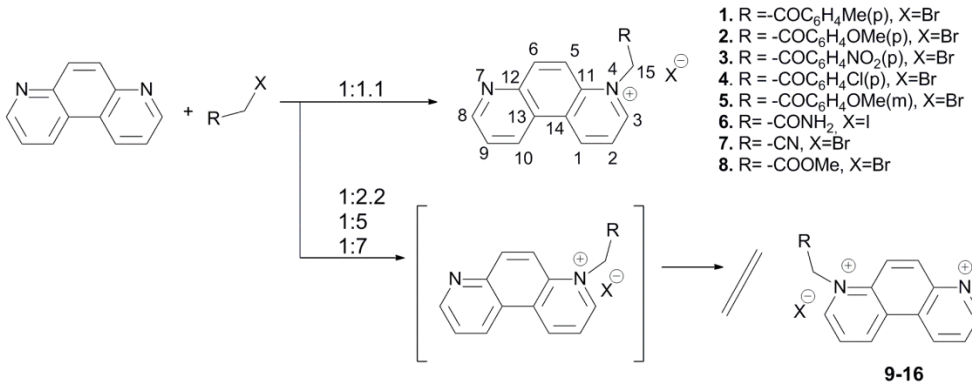
MIC values were reported when the following quality control criteria were satisfied:

- For each plate
  - No growth in the background (un-inoculated) control wells
  - $OD_{590} > 0.3$ in maximum growth wells
  - Rifampicin *MIC* within 3-fold of the expected value
- For each compound curve. *MIC*s were reported if
  - There were 2 points with growth > 75 %
  - There were 2 points with growth < 75 %
- If only one point was > 75 % inhibition then the *MIC* value was reported as the maximum concentration tested
- If no point reached 75 % inhibition, the *MIC* was reported as > maximum concentration tested.

RESULTS AND DISCUSSION

Chemistry

According with our goal, we decided to synthesize new phenanthroline derivatives: 4,7-phenanthrolinium monoquaternary salts (**1**–**8)** and 4,7-phenanthrolinium diquaternary salts (**9**–**16)**. The strategies adopted for the construction of our phenanthroline derivatives is straightforward and efficient, involving an *N*-alkylation reaction of 4,7-phenanthroline, Scheme 1.



Scheme 1. Reaction pathway to obtain 4,7-phenanthrolin-4-ium salts

Unfortunately, no matter the conditions we employed, only 4,7-phenanthrolin-4-ium mono salts, **1**–**8**, have been obtained. As in the case of 1,7- phenanthroline[26], a feasible explanation for this behaviour could be related to the basicity of N_7-nitrogen atom: after the N_4-alkylation of 4,7-phenanthroline, in the obtained phenanthrolin-4-ium mono salts the basicity of N_7-nitrogen decreases, that a second alkylation could not take place.

The salts **1-8** have been prepared in moderate to good yields (58–94 %), using a minimum volume of acetonitrile by refluxing the reaction mixture for 24 hours.

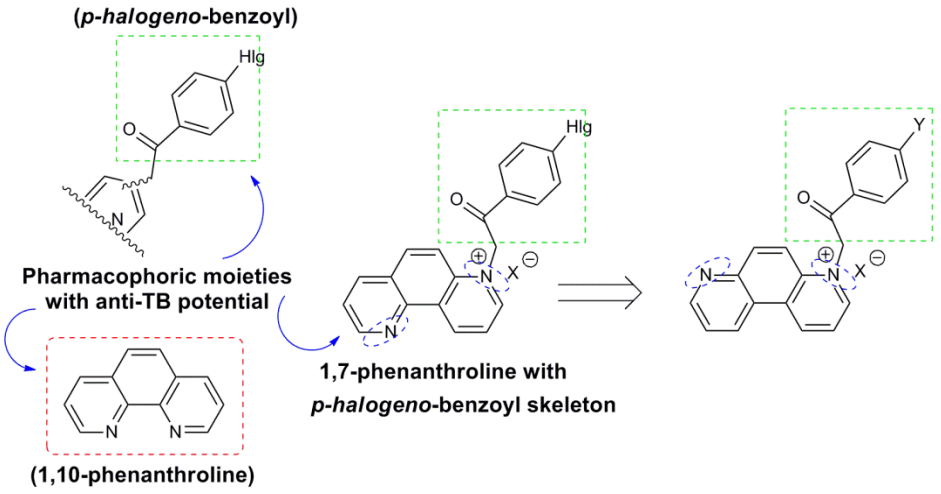
The structure of the new compounds was assigned by elemental and spectroscopic analysis: IR, ^{1}H and ^{13}C NMR. IR spectra of compounds **1**–**6** and **8** are characterized by intense absorption bands in the region of 1672–1698 cm^{-1} specific to C=O stretching, whereas in the IR spectrum of compound **7**, the cyan group furnishes an absorption band at 2201 cm^{-1}. In the ^{1}H NMR spectra of the new monoquaternary salts **1**–**5**, the signals for methylene protons H_{15} appear at low fields (7.07–7.20 ppm, singlet), according with the substituent from the *para* or *meta* position of the benzoyl ring. The same protons appear more shielded (5.27 ppm, 6.58 and 6.35 ppm, respectively) for compounds **6**, **7** and **8**, due to the weaker withdrawing effect of the adjacent carbonyl amide, cyan and ester groups. In the aromatic region, the most unshielded protons are H_3 (those signals appear at 10.22–10.33 ppm) situated in the proximity of the positive nitrogen atom N_4. In the ^{1}H NMR spectra of compound **6**, the two amide protons (NH_2)

furnished two singlet signals at 7.89 and 8.21 ppm, respectively, whereas in the spectrum of compound **8**, the methyl ester protons appear as a singlet at 3.81 ppm. In the ^{13}C-NMR spectra of compounds **1**–**5**, the signals for C_{16} from C=O ketone groups appear at 190.6-188.8 ppm, while for compound **6** having an amide group, respectively for compound **8** with an ester group, appear at 165.8 ppm, and 166.6 ppm, respectively. Methylene C_{15} atoms give signals at 63.7–64.3 ppm for compounds **1**–**5** and at 45.6–59.5 ppm for compounds **6**–**8**. All the other signals from NMR spectra are in agreement with the proposed structures.

Design and Biological Activity

In the continuing battle against *M. tuberculosis*, researchers have found that 1,10-phenanthroline skeleton and (*p*)halogeno-benzoyl moiety are usefully pharmacophoric units for the antimycobacterial activity.[35,36] Moreover, our recent results [26] in the area of 1,7-phenanthrolines salts indicate that, derivatives containing a (*p*)substituted-benzoyl moiety, have activity against *M. tuberculosis* H37Rv, the relative order of activity being (*p*)Cl- > (*p*)Br- > (*p*)methyl-.

Encouraged by these promising results in the field of anti-TB derivatives with phenanthroline skeleton and, especially by our recent results in the area of 1,7-phenanthrolines salts which contain a (*p*)substituted-benzoyl moiety, we decided to combine the biological potentials of 4,7-phenanthroline and (*p*)substituted-benzoyl moiety, intending to obtain compounds with better activity, better pharmacological properties and, to see if changing the nitrogen atoms position of phenanthroline from 1,10-, to more sterically released 4,7-, will affect somehow the activity, Scheme 2. We have had also in view other 4,7-phenanthrolinium salts with –carbalkoxy, cyan and -acetamide moiety, in order to allow structure-activity relationship (SAR) comparisons with (*p*)substituted-benzoyl salts.



Scheme 2. Design in the class of 4,7-phenanthrolin-4-ium derivatives with (*p*)halogeno-benzoyl moiety.

A selection of compounds, salts **1**–**4** and **6**, were evaluated for *in vitro* antimycobacterial activity against *Mycobacterium tuberculosis H37Rv* (grown under aerobic conditions), as a part of our ongoing collaboration with the TAACF TB screening program under direction of the US National Institute of Health, the NIAID division. The MIC of compound was determined by measuring bacterial growth after 5 days in the presence of test compounds.[30-34] The assay is based on measurement of growth in liquid medium of a fluorescent reporter strain of H37Rv where the readout is either optical density (OD) or fluorescence. A linear relationship between OD and fluorescence readout has been established justifying the use of fluorescence as a measure of bacterial growth. MICs generated from the OD are reported in summary data. The MIC was defined as the minimum concentration at which growth was completely inhibited and was calculated from the inflection point of the fitted curve to the lower asymptote (zero growth) (Figure 1A). In addition dose response curves were generated using the Levenberg-Marquardt algorithm and the concentrations that resulted in 50% and 90% inhibition of growth were determined (IC_{50} and IC_{90} respectively) (Figure 1B). The strain has been fully characterized and is equivalent to the parental strain in microbiological phenotypes and virulence. The obtained results are listed in Table I.

TABLE I. Antimycobacterial activity of phenanthrolinium salts **1**–**4** and **6** against *M. tuberculosis* H37Rv under aerobic conditions.

Tested compound	IC_{50} / μM	IC_{90} / μM	*MIC* / μM
1. (R=C_6H_4-Me_p)	110	>200	>200
2. (R=C_6H_4-OMe_p)	>200	>200	>200
3. (R=C_6H_4-NO_{2p})	>200	>200	>200
4. (R=C_6H_4-Cl_p)	83	>200	>200
6. (R=NH_2)	>200	>200	>200
Rifampicin	0.0036	0.0061	0.0055

Also the results are not spectacular, the data from Table I illustrate that two of the five tested compounds have activity against *M. tuberculosis* H37Rv: salt **4** (substituted with (*p*)chloro-benzoyl moiety) and salt **1** (substituted with (*p*)methyl-benzoyl moiety), the first one having a

more pronounced antimycobacterial activity. By comparison with similar 1,7-phenanthrolin-7-ium monoquaternary salts, there is basically only a minor influence of the heterocycle on antimycobacterial activity, the IC_{50} values of the derivatives (1,7 or 4,7-phenanthroline) substituted with the same substituents being similar (*e.g.* for 7-(2-(4-chlorophenyl)-2-oxoethyl-1,7-phenthrolin-7-ium bromide IC_{50} = 88 μM[26]; for 7-(2-oxo-2-(p-tolyl)ethyl)-1,7-phenanthrolin-7-ium bromide IC_{50} = 100 μM[26]).

CONCLUSIONS

The synthesis, structure and *in vitro* antimycobacterial activity of a new class of 4,7-phenanthrolin-4-ium monoquaternary halides is presented. Compounds were prepared by using a straight and efficient method of synthesis. The structure of the new compounds was assigned by elemental and spectroscopic analysis: IR, ^{1}H-NMR and ^{13}C-NMR. The *in vitro* antimycobacterial activity of the synthesized compounds was investigated against *Mycobacterium tuberculosis H37Rv* under aerobic conditions. Two of the five tested compounds have shown activity against *M. tuberculosis* H37Rv, the 4,7-phenanthrolin-7-ium derivative substituted with (*p*)chloro-benzoyl moiety being the most active.

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