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Short communication

SHORT COMMUNICATION

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

CRISTINA M. AL MATARNEH[1], CATALINA I. CIOBANU[2], IONEL I. MANGALAGIU[1] and RAMONA DANAC[1]*

[1]*Alexandru Ioan Cuza University of Iasi, Faculty of Chemistry, Chemistry Department, 11 Carol I, Iasi 700506, Romania and* [2]*Alexandru Ioan Cuza University of Iasi, Faculty of Chemistry, Research Department, 11 Carol I, Iasi 700506, Romania*

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

Keywords: *p*-halogenobenzoyl; cycloimmonium salts; antimycobacterial; 4,7-phenanthroline; *N*-alkylation.

INTRODUCTION

Phenanthroline derivatives have attracted attention especially due to their biological effects,[1–6] crystal engineering,[7–11] 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, such as inhibition of several enzymes,[16–18] microbicide activity especially as an amoebicide[19] and antiviral activity.[20] More

* Corresponding author. E-mail: rdanac@uaic.ro
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recently, 4,7-phenanthroline derivatives were found to stabilize triple-helix DNA[21] and to possess *in vitro* and *in silico* antiviral activity against single-stranded positive-sense RNA genome viruses.[22]

As part of 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 previous promising results in the field of anti-TB derivatives with a nitrogen heterocycle skeleton,[26–30] it was 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) instrument. All chemical shifts are quoted on the δ-scale in ppm. Coupling constants are given in Hz. IR spectra were recorded on a Shimadzu Prestige 8400S FTIR 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**)*

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

The following compounds were synthesized: 4-(2-oxo-2-*p*-tolylethyl)-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**) and 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

The compounds were evaluated for their 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. The evaluation of the antimycobacterial activities of the compounds were performed at the Center of Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) of the Southern Research Institute.[31-34]

Primary cycle high throughput screening (HTS). Determination of the 90 % inhibitory concentration (IC_{90}), 50 % inhibitory concentration (IC_{50}) and minimum inhibitory concentration (MIC)

The *MIC* values of the compounds were determined by measuring the 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 the compounds was

200 μM when the compounds were soluble in DMSO at 10 mM. For compounds with limited solubility, the highest concentration was 50× less than the stock concentration, *e.g.*, 100 μM for 5 mM DMSO stock and 20 μM for 1 mM DMSO stock. For potent compounds, the 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. The plates were inoculated with *M. tuberculosis* and incubated for 5 days: growth was measured by the optical density at 590 nm (OD_{590}) and fluorescence (Ex. 560 nm/Em. 590 nm) using a BioTek™ Synergy 4 plate reader. Growth was calculated separately for OD_{590} and relative fluorescence units (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 the Gompertz model 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).

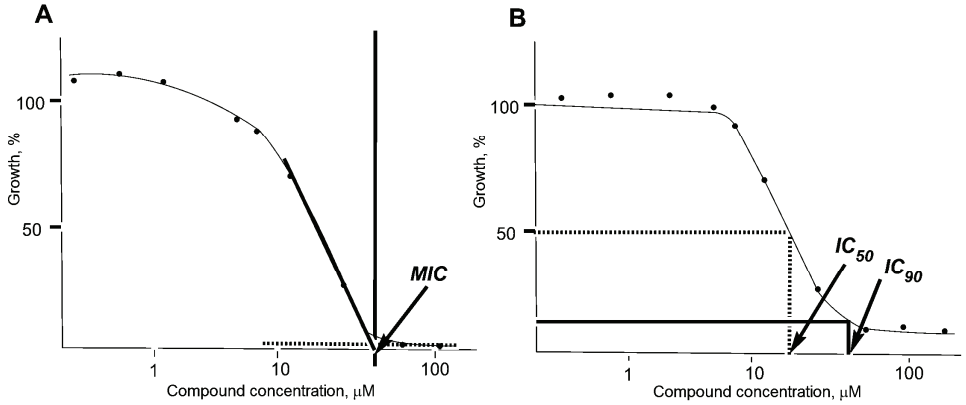


Fig. 1. Dose response curves used to calculate the *MIC*, IC_{50} and IC_{90}.

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.

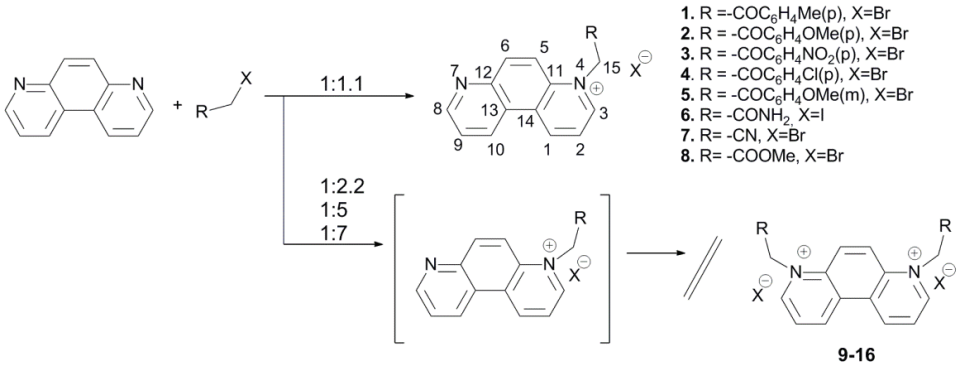
– For each compound curve, the *MIC*s were reported if there were 2 points with growth >75 %. If only one point was >75 % inhibition, then the *MIC* value was reported as the maximum concentration tested. If no point reached 75 % inhibition, the *MIC* was reported as >maximum concentration tested.

RESULTS AND DISCUSSION

Chemistry

According with the goal of this study, it was 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 con-

struction of the phenanthroline derivatives was straightforward and efficient, involving an *N*-alkylation reaction of 4,7-phenanthroline, Scheme 1.



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

Unfortunately, it was not possible to synthesize compounds **9**–**16**, under any of the employed conditions (ambient temperature or refluxing, content of 4,7--phenanthroline. Thus, on varying the amount of the reactive halides from 1:2 to 1:7, and using different solvents, *i.e*,. acetone, acetonitrile and dimethylformamide, only 4,7-phenanthrolin-4-ium mono salts **1**–**8** were obtained.

As in the case of 1,7-phenanthroline,[26] a feasible explanation for this behavior could be related to the basicity of the N7-nitrogen atom, *i.e*., after the N4-alkylation of 4,7-phenanthroline, the basicity of the N7-nitrogen is decreased in the obtained phenanthrolin-4-ium mono salts so that the second alkylation could not occur.

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

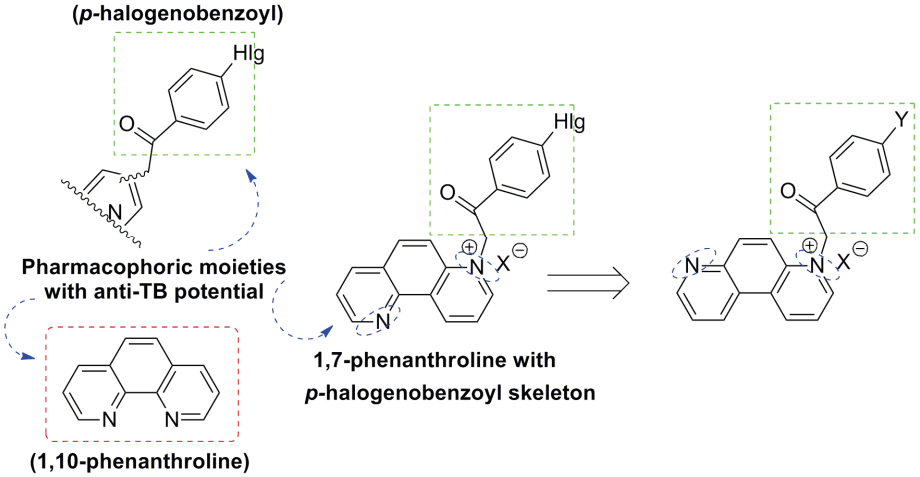
The structure of the new compounds was assigned by elemental and spectroscopic analysis: IR, ^{1}H- and ^{13}C-NMR. The IR spectra of compounds **1**–**6** and **8** are characterized by intense absorption bands in the region of 1672–1698 cm^{-1} specific to C=O stretching, whereas in the IR spectrum of compound **7** the cyano 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 H15 appear at low fields (7.07–7.20 ppm, singlet), according to the substituent on the *para* or *meta* position of the benzoyl ring. The same protons appear more shielded at 5.27, 6.58 and 6.35 ppm for compounds **6**–**8**, respectively, due to the weaker withdrawing effect of the adjacent carbonyl amide, cyano and ester groups. In the aromatic region, the most unshielded protons are H3 (the signals of which appear at 10.22–10.33 ppm) situated in the proximity of the positive nitrogen atom N4. 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, 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 C16 from the C=O ketone groups appear at 190.6–188.8 ppm, while for compound **6**, having an amide group, and for compound **8** with an ester group, it appears at 165.8 and 166.6 ppm, respectively. The methylene C15 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 the 1,10-phenanthroline skeleton and a *p*-halogenobenzoyl moiety are useful pharmacophoric units for the antimycobacterial activity.[35,36] Moreover, recent results[26] in the area of 1,7-phenanthroline salts indicate that derivatives containing a *p*-substituted benzoyl moiety exhibit 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 the phenanthroline skeleton and, especially by recent results in the area of 1,7-phenanthroline salts which contain a *p*-substituted-benzoyl moiety, it was decided to combine the biological potentials of 4,7-phenanthroline and the *p*-substituted benzoyl moiety, with the intention of obtaining compounds with better activity and better pharmacological properties, and to determine whether changing the nitrogen atoms position of phenanthroline from 1,7- to 4,7- would somehow affect the activity, Scheme 2. Also in view were other 4,7-phenanthrolinium salts with the alkoxycarbonyl, cyano 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 *M. tuberculosis* H37Rv (grown under aerobic conditions), as a part of ongoing collaboration with the TAACF TB screening program under direction of the US National Institute of Health, the NIAID division. The *MIC* value were determined by measuring bacterial growth after 5 days in the presence of the test compounds.[30–34] The assay was based on measurement of the growth in liquid medium of a fluorescent reporter strain of H37Rv where the readout was either optical density (*OD*) or fluorescence. A linear relationship between the *OD* and fluorescence readout was established, justifying the use of fluorescence as a measure of bacterial growth. The *MIC* values generated from the *OD* measurements are reported in the 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, 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). 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

Although the results are not spectacular, the data from Table I illustrate that two of the five tested compounds exhibited activity against *M. tuberculosis* H37Rv: salt **4** (substituted with *p*-chlorobenzoyl moiety) and salt **1** (substituted with *p*-methylbenzoyl moiety), the first one having a more pronounced antimycobacterial activity. By comparison with similar 1,7-phenanthrolin-7-ium monoquaternary salts it could be seen that the heterocycle has only a minor influence of 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], while for 7-(2-oxo-2-*p*-tolylethyl)-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 are presented. The compounds were prepared by a straightforward and efficient method. 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 *M. tuberculosis* H37Rv under aerobic conditions. Two of the five tested compounds showed activity against *M. tuberculosis* H37Rv, the 4,7-phenanthrolin-7-ium derivative substituted with *p*-chloro-benzoyl moiety being the most active.

SUPPLEMENTARY MATERIAL

The physical and spectral data for the prepared compounds are available electronically from http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

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ИЗВОД

ДИЗАЈН, СИНТЕЗА И ИСПИТИВАЊЕ АНТИМИКОБАКТЕРИЈСКЕ АКТИВНОСТИ НОВИХ АЗАХЕТЕРОЦИКЛА СА 4,7-ФЕНАНТРОЛИНСКИМ СКЕЛЕТОМ. 6. ДЕО

CRISTINA M. AL MATARNEH[1], CATALINA I. CIOBANU[2], IONEL I. MANGALAGIU[1] и RAMONA DANAC[1]

[1]*Alexandru Ioan Cuza University of Iasi, Faculty of Chemistry, Chemistry Department, 11 Carol I, Iasi 700506, Romania и* [2] *Alexandru Ioan Cuza University of Iasi, Faculty of Chemistry, Research Department, 11 Carol I, Iasi 700506, Romania*

Приказано је истраживање у ком је описана синтеза, одређивање структуре и *in vitro* испитивање антимикобактеријске активности нових деривата 4,7-фенантролина. Синтеза деривата је директна и ефикасна и укључује реакције *N*-алкиловања 4,7-фенантролина. Структура добијених деривата утврђена је елементалном анализом и спектроскопским методама (IR, ^{1}H- и ^{13}C-NMR). Антимикобактеријска *in vitro* активност пет синтетисаних једињења испитана је према *Mycobacterium tuberculosis* H37Rv под аеробним условима. Утврђено је да постоји утицај супституената у *ūара* положају бензоил језгра и да 4,7-фенантролин-4-ијум со са *p*-хлорбензоил групом показује веома изражену антимикобактеријску активност.

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