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RESEARCH ARTICLE

Synthesis, structure, antimycobacterial and anticancer evaluation of new pyrrolo-phenanthroline derivatives

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Abstract

A study concerning design, synthesis, structure and *in vitro* antimycobacterial and anticancer evaluation of new fused derivatives with pyrrolo[2,1-*c*][4,7]phenanthroline skeleton is described. The strategy adopted for synthesis involves a [3 + 2] dipolar cycloaddition of several *in situ* generated 4,7-phenanthrolin-4-ium ylides to different substituted alkynes and alkenes. Stereo- and regiochemistry of cycloaddition reactions were discussed. The structure of the new compounds was proven unambiguously, single-crystal X-ray diffraction studies including. The antimycobacterial and anticancer activity of a selection of new synthesized compounds was evaluated against *Mycobacterium tuberculosis H37Rv* under aerobic conditions and 60 human tumour cell line panel, respectively. Five of the tested compounds possess a moderate antimycobacterial activity, while two of the compounds have a significant antitumor activity against renal cancer and breast cancer.

Keywords

3 + 2 Cycloadditions, N-ylides, pyrrolo[2,1-*c*][4,7]phenanthrolines

History

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Introduction

Phenanthroline derivatives attracted attention in the last years especially due to their biological effects[1–3], materials science applications[4], crystal engineering[5,6], their unique π-electrons delocalization[7,8] and complexation properties[9].

Phenanthrolines polycyclic skeletons are also present in sterols, sex hormones, cardiac glycosides, bile acids and morphine alkaloids[10]. Recently, compounds with phenathroline skeleton have been synthesized and biologically evaluated[11–15] as analogues of benzo[c]phenanthridine alkaloids (e.g. nitidine, fagaronine, sanguinarine) that attracted attention due to their interesting biological properties[16–19].

Compared to 1,10-phenanthroline that has been widely studied both for synthesis and applications, 4,7-phenanthroline received much less interest due to difficulties in its synthesis. However, there are several reports regarding biological properties of 4,7-phenanthroline derivatives and their analogues as antibacterial activity[20], amoebicide[21] or antiviral properties[22]. Besides, 4,7-phenathroline derivatives were found to exhibit staining properties to HeLa cells and can be used as fluorophores which can bind with proteins[23]. Other derivatives were reported as high affinity triple-helix DNA stabilizing agents[24].

As part of our ongoing research in the field of design and synthesis of new anti-TB[25–28] and anticancer derivatives[29–32] with azaheterocycles skeleton, we report here the design, synthesis, structure and *in vitro* antimycobacterial and anticancer evaluation of new class of compounds with pyrrolo[2,1-*c*][4,7]phenanthroline scaffold. To the best of our knowledge, there is no previous report, concerning the synthesis of pyrrolo[2,1-*c*][4,7]-phenanthroline derivatives.

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Methods

General

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 or Jasco 660 *plus* FTIR spectrophotometer. Thin layer chromatography (TLC) was carried out on Merck silica gel $60F_{254}$ plates. Column chromatography was carried out on silica gel (Roth 60, 0.04–0.063 mm). Visualization of the plates was achieved using a UV lamp ($\lambda_{max} = 254$ or 365 nm). All commercially available products were used without further purification unless otherwise specified.

X-ray crystallography

Crystallographic measurements were carried out with an Oxford-Diffraction XCALIBUR E CCD diffractometer equipped with graphite-monochromated Mo *Kα* radiation. Single crystals were positioned at 40 mm from the detector and 211 and 248 frames were measured each for 60 and 180 s over 1 ° scan width for 2259 and 2340, respectively. The unit cell determination and data integration were carried out using the CrysAlis package of Oxford Diffraction[33]. The structures were solved by direct methods using Olex2[34] software with the SHELXS structure solution program and refined by full-matrix least-squares on F^2 with SHELXL-97[35]. Atomic displacements for non-hydrogen atoms were refined

using an anisotropic model. Hydrogen atoms were placed in fixed, idealized positions and refined as rigidly bonded to the corresponding atoms. The molecular plots were obtained using the Olex2 program. The main crystallographic data together with refinement details are summarized in Supplemental Table S1. CCDC-1048147 (for **7a**) and 1048148 (for **11f**) contain the supplementary crystallographic data for this contribution. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or deposit@ccdc.ca.ac.uk).

General procedure for synthesis of compounds 4, 6, 7, 8, 9, 10 and 11

The cycloimmonium salt **1a–e** (1 mmol, 1 equiv.) and dipolarophile (DMAD, EP, N-phenylmaleimide or acrylonitrile, 1.1 mmol, 1.1 equiv.) were added to 5 mL of dichloromethane and the obtained suspension is stirred at room temperature (rt) under N_2 atmosphere. Triethylamine (TEA, 3 mmol, 3 equiv.) was added drop-wise over 1 h (magnetic stirring) and the resulting mixture was then stirred for 24 h at rt under nitrogen. Methanol (5 mL) was added and the resulting mixture is kept for 24 h without stirring. The formed precipitate was collected by filtration to give a powder which was washed with few mL of methanol. The product was crystallized from an appropriate solvent or purified by column chromatography.

*Dimethyl 9-(4-methoxybenzoyl)pyrrolo[2,1-c][4,7]phenanthroline-7,8-dicarboxylate (**4a**)*

Crystallized from ethanol–chloroform 1:1, (v/v). Yellow powder (51% yield), mp = 244–247 °C. IR [KBr, ν (cm^{-1})]: 2953, 1739, 1702, 1596, 1252, 1211, 1161, 1086, 803. ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): δ 3.53 (s, 3H, 21-H), 3.90 (s, 3H, OMe), 3.91 (s, 3H, 19-H), 6.99 (d, J = 8.5 Hz, 2H, phenyl-H), 7.57 (dd, J = 8.0, 4.0 Hz, 1H, 3-H), 7.92 (d, J = 9.5 Hz, 1H, 11-H), 8.02 (d, J = 8.5 Hz, 2H, phenyl-H), 8.07 (d, J = 9.5 Hz, 1H, 12-H), 8.29 (d, J = 9.5 Hz, 1H, 5-H), 8.37 (d, J = 9.5 Hz, 1H, 6-H), 8.80 (d, J = 8.0 Hz, 1H, 4-H), 8.95 (d, J = 4.0 Hz, 1H, 2-H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 51.9 C-19, 52.6 C-21, 55.8 OMe, 105.0 C-7, 114.2 2 × CH-Ph, 118.9 C-6, 120.9 C-14, 121.7 C-5, 122.0 C-11, 122.5 C-3, 125.0 C-13, 126.3 C-9, 128.6 C-8, 130.4 Cq-Ph, 130.9 C-12, 131.0 C-16, 131.3 C-4, 132.6 2 × CH-Ph, 137.0 C-15, 146.0 C-17, 150.6 C-2, 163.6 C-18, 164.7 Cq-Ph, 165.4 C-20, 186.2 CO ppm. Anal. Calcd. for $C_{27}H_{20}N_2O_6$: C, 69.22; H, 4.30; N, 5.98. Found: C, 69.39, H, 4.28; N, 6.12.

*Dimethyl 9-(4-nitrobenzoyl)pyrrolo[2,1-c][4,7]phenanthroline-7,8-dicarboxylate (**4b**)*

Crystallized from ethanol–chloroform 1:1, (v/v). Orange powder (50% yield), mp = 237–238 °C. IR [KBr, ν(cm^{-1})): 1736, 1718, 1699, 1488, 1340, 1235, 1203, 1091, 805. ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): δ 3.48 (s, 3H, 21-H), 3.94 (s, 3H, 19-H), 7.68 (dd, J = 8.5, 4.5 Hz, 1H, 3-H), 7.87 (d, J = 9.5 Hz, 1H, 11-H), 8.16 (d, J = 8.5 Hz, 3H, phenyl-H, 12-H), 8.36 (d, J = 8.5 Hz, 2H, phenyl-H), 8.51 (d, J = 9.5 Hz, 1H, 5-H), 8.57 (d, J = 9.5 Hz, 1H, 6-H), 8.91 (d, J = 8.5 Hz, 1H, 4-H), 9.05 (d, J = 4.5 Hz, 1H, 2-H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 52.2 C-19, 52.8 C-21, 105.9 C-7, 118.9 C-6, 121.4 C-14, 122.1 C-11, 122.8 C-3, 123.3 C-5, 123.9 2 × CH-Ph, 125.0 C-9, 125.1 C-13, 130.0 C-8, 130.9 2 × CH-Ph, 131.0 C-16, 131.0 C-4, 131.5 C-12, 137.7 C-15, 142.6 Cq-Ph, 146.2 C-17, 150.8 Cq-Ph, 151.0 C-2, 163.3 C-18, 165.2 C-20, 184.5 CO ppm. Anal. Calcd. for $C_{26}H_{17}N_3O_7$: C, 64.60; H, 3.54; N, 8.69. Found: C, 64.69, H, 3.48; N, 8.83.

*Dimethyl 9-(4-chlorobenzoyl)pyrrolo[2,1-c][4,7]phenanthroline-7,8-dicarboxylate (**4c**)*

Crystallized from ethanol–chloroform 1:1, (v/v). Yellow powder (55% yield), mp = 245–247 °C. IR [KBr, ν (cm^{-1})]: 2947, 1726, 1710, 1644, 1223, 1212, 1086. ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): δ 3.51 (s, 3H, 21-H), 3.93 (s, 3H, 19-H), 7.50 (d, J = 8.0 Hz, 2H, phenyl-H), 7.65 (dd, J = 8.0, 4.0 Hz, 1H, 3-H), 7.89 (d, J = 9.5 Hz, 1H, 11-H), 7.96 (d, J = 8.0 Hz, 2H, phenyl-H), 8.12 (d, J = 9.5 Hz, 1H, 12-H), 8.44 (d, J = 9.5 Hz, 1H, 5-H), 8.55 (d, J = 9.5 Hz, 1H, 6-H), 8.88 (d, J = 8.0 Hz, 1H, 4-H), 9.03 (as, 1H, 2-H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 52.0 C-19, 52.7 C-21, 105.4 C-7, 118.9 C-6, 121.2 C-14, 122.1 C-11, 122.5 C-5, 122.7 C-3, 125.1 C-13, 125.6 C-9, 129.2 2 × CH-Ph, 130.1 C-8, 131.0 C-16, 131.2 C-4, 131.3 C-12, 131.4 2 × CH-Ph, 136.1 Cq-Ph, 137.7 C-15, 140.7 Cq-Ph, 146.2 C-17, 150.9 C-2, 163.5 C-18, 165.3 C-20, 185.8 CO ppm. Anal. Calcd. for $C_{26}H_{17}ClN_2O_5$: C, 66.04; H, 3.62; N, 5.92. Found: C, 66.20, H, 3.53; N, 5.99.

*Dimethyl 9-cyanopyrrolo[2,1-c][4,7]phenanthroline-7,8-dicarboxylate (**4d**)*

Crystallized from ethanol–chloroform 1:1, (v/v). Brown powder (50% yield), mp = 261–263 °C. IR [KBr, ν (cm^{-1})]: 2955, 2216, 1740, 1694, 1225, 1099. ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): δ 3.97 (s, 3H, 19-H), 4.07 (s, 3H, 21-H), 7.71 (dd, J = 8.5, 4.0 Hz, 1H, 3-H), 8.42 (d, J = 10.0 Hz, 1H, 12-H), 8.46 (d, J = 10.0 Hz, 1H, 5-H), 8.51 (d, J = 10.0 Hz, 1H, 6-H), 8.92 (d, J = 8.5 Hz, 1H, 4-H), 9.11 (ad, J = 4.0 Hz, 1H, 2-H), 9.51 (d, J = 10.0 Hz, 1H, 11-H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 52.3 C-19, 53.4 C-21, 98.3 C-9, 106.9 C-7, 114.7 CN, 118.1 C-11, 118.9 C-6, 121.1 C-14, 122.5 C-5, 123.0 C-3, 125.1 C-13, 131.4 C-4, 132.0 C-16, 133.4 C-8, 132.7 C-12, 136.8 C-15, 146.4 C-17, 151.4 C-2, 162.7 C-18, 163.3 C-20 ppm. Anal. Calcd. for $C_{20}H_{13}N_3O_4$: C, 66.85; H, 3.65; N, 11.69. Found: C, 66.81, H, 3.58; N, 11.72.

*Trimethyl pyrrolo[2,1-c][4,7]phenanthroline-7,8,9-tricarboxylate (**4e**)*

Crystallized from ethanol–chloroform 1:1, (v/v). Pale pink powder (55% yield), mp = 215–218 °C. IR [KBr, ν (cm^{-1})]: 2953, 1741, 1704, 1677, 1263, 1161, 1099, 802. ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): δ 3.94 (s, 3H, 19-H), 4.00 (s, 3H, OMe), 4.03 (s, 3H, 21-H), 7.76 (dd, J = 8.0, 4.0 Hz, 1H, 3-H), 8.36 (d, J = 10.0 Hz, 1H, 11-H), 8.38 (d, J = 9.5 Hz, 1H, 5-H), 8.43 (d, J = 10.0 Hz, 1H, 12-H), 8.49 (d, J = 9.5 Hz, 1H, 6-H), 9.01 (d, J = 8.5 Hz, 1H, 4-H), 9.05 (dd, J = 4.0, 1.0 Hz, 1H, 2-H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 52.1 C-19, 52.9 OMe, 53.2 C-21, 105.4 C-7, 117.6 C-9, 119.3 C-6, 121.1 C-14, 122.2 C-5, 122.5 C-3, 124.1 C-11, 125.2 C-13, 128.3 C-12, 131.6 C-16, 132.3 C-8, 133.6 C-4, 137.9 C-15, 144.1 C-17, 148.7 C-2, 161.1 COOMe, 163.2 C-18, 165.9 C-20 ppm. Anal. Calcd. for $C_{21}H_{16}N_2O_6$: C, 64.28; H, 4.11; N, 7.14. Found: C, 64.20, H, 4.03; N, 7.20.

*Ethyl 9-(4-methoxybenzoyl)pyrrolo[2,1-c][4,7]phenanthroline-7-carboxylate (**6a**)*

Purified by column chromatography: CH_2Cl_2→ CH_2Cl_2: MeOH (98:2, v:v), then crystallized from ethanol–chloroform 1:1, (v/v). Orange powder (50% yield), mp = 252–253 °C. IR [KBr, ν (cm^{-1})]: 2982, 1694, 1232, 1166, 1080, 800. ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): δ 1.41 (t, J = 7.0 Hz, 3H, 20-H), 3.95 (s, 3H, OMe), 4.40 (q, 2H, J = 7.0 Hz, 19-H), 7.07 (d, J = 8.0 Hz, 2H, phenyl-H), 7.60 (dd, J = 8.0, 4.0 Hz, 1H, 3-H), 7.73 (s, 1H, 8-H), 8.20 (m, 4H, 11-H, 12-H, phenyl-H), 8.40 (d, J = 9.5 Hz, 1H, 5-H), 8.51 (d, J = 9.5 Hz, 1H, 6-H), 8.85 (d, J = 8.0 Hz, 1H, 4-H), 8.99 (d, J = 4.0 Hz, 1H, 2-H). ^{13}C NMR (125 MHz, $CDCl_3$):

δ 14.7 C-20, 55.8 OMe, 60.4 C-19, 107.4 C-7, 114.1 2 × CH-Ph, 118.6 C-6, 120.6 C-14, 122.3 C-3, 122.4 C-5, 123.3 C-11, 125.0 C-13, 128.0 C-9, 129.1 C-8, 130.8 Cq-Ph, 130.6 C-12, 131.2 C-4, 131.7 C-16, 132.6 2 × CH-Ph, 139.8 C-15, 146.3 C-17, 150.4 C-2, 163.9 Cq-Ph, 164.2 C-18, 184.0 CO ppm. Anal. Calcd. for $C_{26}H_{20}N_2O_4$: C, 73.57; H, 4.57; N, 6.60. Found: C, 73.52, H, 4.49; N, 6.62.

*Ethyl 9-(4-nitrobenzoyl)pyrrolo[2,1-c][4,7]phenanthroline-7-carboxylate (**6b**)*

Crystallized from ethanol–chloroform 1:1, (v/v). Orange powder (49% yield), mp = 332–335 °C. IR [KBr, *v* (cm^{-1})]: 3070, 2968, 1694, 1638, 1516, 1495, 1344, 1237, 1081, 801. ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): δ 1.42 (t, *J* = 7.0 Hz, 3H, 20-H), 4.41 (q, 2H, *J* = 7.0 Hz, 19-H), 7.68 (dd, *J* = 8.5, 4.0 Hz, 1H, 3-H), 7.75 (s, 1H, 8-H), 8.29 (m, 4H, 11-H, 12-H, phenyl-H), 8.44 (d, *J* = 8.5 Hz, 2H, phenyl-H), 8.59 (d, *J* = 9.5 Hz, 1H, 5-H), 8.63 (d, *J* = 9.5 Hz, 1H, 6-H), 8.94 (d, *J* = 8.5 Hz, 1H, 4-H), 9.06 (d, *J* = 4.0 Hz, 1H, 2-H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.7 C-20, 60.7 C-19, 108.4 C-7, 118.6 C-6, 121.1 C-14, 122.6 C-3, 123.4 C-11, 124.0 2 × CH-Ph, 124.1 C-5, 125.0 C-13, 127.4 C-9, 131.0 2 × CH-Ph, 131.4 C-12, 131.5 C-4, 131.6 C-8, 131.9 C-16, 141.2 C-15, 143.8 Cq-Ph, 146.5 C-17, 150.4 Cq-Ph, 150.8 C-2, 163.7 C-18, 181.8 CO ppm. Anal. Calcd. for $C_{25}H_{17}N_3O_5$: C, 68.33; H, 3.90; N, 9.56. Found: C, 68.35, H, 3.80; N, 9.63.

*Ethyl 9-(4-chlorobenzoyl)pyrrolo[2,1-c][4,7]phenanthroline-7-carboxylate (**6c**)*

Crystallized from ethanol–chloroform 1:1, (v/v). Yellow powder (59% yield), mp = 278–280 °C. IR [KBr, *v* (cm^{-1})]: 3049, 2977, 1697, 1229, 1211, 1077, 803. ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): δ 1.42 (t, *J* = 7.0 Hz, 3H, 20-H), 4.40 (q, 2H, *J* = 7.0 Hz, 19-H), 7.57 (d, *J* = 8.0 Hz, 2H, phenyl-H), 7.62 (dd, *J* = 8.0, 4.0 Hz, 1H, 3-H), 7.73 (s, 1H, 8-H), 8.09 (d, *J* = 8.0 Hz, 2H, phenyl-H), 8.22 (as, 2H, 11-H, 12-H), 8.46 (d, *J* = 9.5 Hz, 1H, 5-H), 8.54 (d, *J* = 9.5 Hz, 1H, 6-H), 8.88 (d, *J* = 8.0 Hz, 1H, 4-H), 9.01 (d, *J* = 4.0 Hz, 1H, 2-H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.7 C-20, 60.5 C-19, 107.8 C-7, 118.5 C-6, 120.8 C-14, 122.4 C-3, 123.2 C-5, 123.4 C-11, 125.0 C-13, 127.6 C-9, 129.1 2 × CH-Ph, 130.4 C-8, 130.8 C-12, 131.3 C-4, 131.6 2 × CH-Ph, 131.8 C-16, 136.7 Cq-Ph, 139.6 Cq-Ph, 140.5 C-15, 146.3 C-17, 150.6 C-2, 164.0 C-18, 185.3 CO ppm. Anal. Calcd. for $C_{25}H_{17}ClN_2O_3$: C, 70.01; H, 4.00; N, 6.53. Found: C, 69.99, H, 3.93; N, 6.60.

*Ethyl 9-cyanopyrrolo[2,1-c][4,7]phenanthroline-7-carboxylate (**6d**)*

Crystallized from ethanol–chloroform 1:1, (v/v). Brown powder (55% yield), mp = 224–226 °C. IR [KBr, *v* (cm^{-1})]: 3075, 2992, 2213, 1698, 1248, 1080, 799. ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): δ 1.45 (t, *J* = 7.0 Hz, 3H, 20-H), 4.43 (q, 2H, *J* = 7.0 Hz, 19-H), 7.67 (dd, *J* = 8.5, 4.0 Hz, 1H, 3-H), 7.96 (s, 1H, 8-H), 8.37 (d, *J* = 9.5 Hz, 1H, 12-H), 8.40 (d, *J* = 10.0 Hz, 1H, 5-H), 8.58 (d, *J* = 10.0 Hz, 1H, 6-H), 8.90 (d, *J* = 8.5 Hz, 1H, 4-H), 9.07 (ad, *J* = 4.0 Hz, 1H, 2-H), 9.46 (d, *J* = 9.5 Hz, 1H, 11-H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.6 C-20, 60.7 C-19, 97.7 C-9, 108.3 C-7, 115.8 CN, 118.2 C-11, 118.9 C-6, 120.5 C-14, 121.6 C-5, 122.8 C-3, 125.1 C-13, 128.7 C-8, 131.3 C-4, 132.3 C-12, C-16, 137.5 C-15, 146.2 C-17, 151.1 C-2, 163.2 C-18 ppm. Anal. Calcd. for $C_{19}H_{13}N_3O_2$: C, 72.37; H, 4.16; N, 13.33. Found: C, 72.35, H, 4.09; N, 13.39.

*7-Ethyl 9-methyl pyrrolo[2,1-c][4,7]phenanthroline-7, 9-dicarboxylate (**6e**)*

Crystallized from ethanol–chloroform 1:1, (v/v). Pale pink powder (49% yield), mp = 173–174 °C. IR [KBr, *v* (cm^{-1})]: 3076, 2993, 2213, 1726, 1689, 1248, 1080, 799. ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): δ 1.44 (t, *J* = 7.0 Hz, 3H, 20-H), 4.00 (s, 3H, OMe), 4.41 (q, 2H, *J* = 7.0 Hz, 19-H), 7.57 (dd, *J* = 8.5, 4.0 Hz, 1H, 3-H), 8.05 (s, 1H, 8-H), 8.21 (d, *J* = 10.0 Hz, 1H, 12-H), 8.29 (d, *J* = 9.5 Hz, 1H, 5-H), 8.45 (d, *J* = 9.5 Hz, 1H, 6-H), 8.54 (d, *J* = 10.0 Hz, 1H, 11-H), 8.98 (d, *J* = 8.5 Hz, 1H, 4-H), 8.98 (ad, *J* = 4.0 Hz, 1H, 2-H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.7 C-20, 52.4 OMe, 60.4 C-19, 107.4 C-7, 118.6 C-6, 119.7 C-9, 120.7 C-14, 121.6 C-5, 122.2 C-3, 123.2 C-11, 124.8 C-13, 127.5 C-8, 129.9 C-12, 131.3 C-4, 131.8 C-16, 139.5 C-15, 146.0 C-17, 150.3 C-2, 162.2 COOMe, 164.0 C-18 ppm. Anal. Calcd. for $C_{20}H_{16}N_2O_4$: C, 68.96; H, 4.63; N, 8.04. Found: C, 68.90, H, 4.57; N, 8.10.

*Ethyl 9-(4-methoxybenzoyl)-8,9-dihydropyrrolo[2,1-c][4,7] phenanthroline-7-carboxylate (**7a**)*

Purified by column chromatography: CH_2Cl_2→ CH_2Cl_2: MeOH (98:2, v:v), then crystallized from ethanol–chloroform 1:1, (v/v). Red crystals (25% yield), mp = 243–246 °C. IR [KBr, *v* (cm^{-1})]: 1684, 1650, 1266, 1222, 1163, 1060, 1033, 803. ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): δ 1.28 (t, *J* = 7.0 Hz, 3H, 20-H), 3.09 (dd, *J* = 15.5, 5.5 Hz, 1H, 8-H), 3.64 (at, *J* = 9.5 Hz, 8-H), 3.93 (s, 3H, OMe), 4.18 (q, 2H, *J* = 7.0 Hz, 19-H), 6.88 (d, *J* = 9.5 Hz, 11-H), 6.99 (dd, *J* = 14.0, 5.0 Hz, 1H, 9-H), 7.04 (d, *J* = 8.5 Hz, 2H, phenyl-H), 7.48 (dd, *J* = 8.5, 4.0 Hz, 1H, 3-H), 7.73 (d, *J* = 10.0 Hz, 1H, 5-H), 7.92 (d, *J* = 10.0 Hz, 1H, 6-H), 7.97 (d, *J* = 9.5 Hz, 12-H), 8.04 (d, *J* = 8.5 Hz, 2H, phenyl-H), 8.49 (d, *J* = 8.5 Hz, 1H, 4-H), 8.77 (d, *J* = 4.0 Hz, 1H, 2-H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.9 C-20, 33.7 C-8, 55.8 OMe, 59.0 C-19, 64.3 C-9, 90.1 C-7, 114.6 2 × CH-Ph, 117.3 C-11, 119.2 C-5, 115.2 C-14, 122.6 C-3, 124.8 C-13, 125.8 Cq-Ph, 129.5 C-6, 130.0 C-4, 130.1 C-16, 131.5 2 × CH-Ph, 132.8 C-12, 138.0 C-15, 144.0 C-17, 148.2 C-2, 164.7 Cq-Ph, 166.1 C-18, 191.5 CO ppm. Anal. Calcd. for $C_{26}H_{22}N_2O_4$: C, 73.23; H, 5.20; N, 6.57. Found: C, 73.22, H, 5.09; N, 6.62.

*12-(4-Nitrobenzoyl)-10-phenyl-11a,12-dihydro-8aH-pyrrolo[3′,4′:3,4]pyrrolo[2,1-c][4,7]phenanthroline-9,11(8bH,10H)-dione (**8b**)*

Crystallized from ethanol-chloroform 1:1, (v/v). Yellow powder (58% yield), mp = 177–180 °C. IR [KBr, *v* (cm^{-1})]: 1718, 1596, 1523, 1497, 1382, 1343, 1195. ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): δ 3.72 (t, *J* = 8.0 Hz, 1H, 8-H), 3.84 (ad, *J* = 8.0 Hz, 1H, 18-H), 5.39 (d, *J* = 8.0, Hz, 1H, 7-H), 5.99 (s, 1H, 12-H), 6.28 (ad, *J* = 10.0 Hz, 1H, 6-H), 6.79 (d, *J* = 9.0 Hz, 1H, 13-H), 7.16 (ad, *J* = 8.0 Hz, 3H, 5-H, 2 × phenyl-CH), 7.35-7.43 (m, 4H, 3 × phenyl-CH, 3-H), 7.86 (d, *J* = 9.0 Hz, 1H, 14-H), 8.29 (ad, *J* = 9.0 Hz, 1H, 4-H), 8.45 (d, *J* = 8.5 Hz, 2H, 2 × CH-(*p*)-NO_2-Ph, 8.54 (d, *J* = 8.5 Hz, 2H, 2 × CH-(*p*)-NO_2-Ph, 8.69 (ad, *J* = 3.0 Hz, 1H, 2-H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 46.7 C-18, 48.1 C-8, 61.1 C-7, 65.5 C-12, 114.0 C-17, 116.9 C-13, 120.1 C-6, 121.7 C-5, 121.9 C-3, 124.6 2 × CH-(*p*)-NO_2-phenyl, 125.8 C-16, 126.3 2 × CH-Ph, 129.3 CH-Ph, 129.4 2 × CH-Ph, 129.7 C-4, 130.5 2 × CH-(*p*)-NO_2-phenyl, 130.9 C-14, 131.4 Cq-Ph, 137.6 Cq-(*p*)-NO_2-phenyl, 139.5 C-19, 142.0 C-15, 147.4 C-2, 151.3 C-NO_2, 174.4 C-9, 176.2 C-11, 194.1 C-20 ppm. Anal. Calcd. for $C_{30}H_{20}N_4O_5$: C, 69.76; H, 3.90; N, 10.85. Found: C, 69.77, H, 3.82; N, 10.95.

*12-(3-Methoxybenzoyl)-10-phenyl-11a,12-dihydro-8aH-pyrrolo[3′,4′:3,4]pyrrolo[2,1-c][4,7]phenanthroline-9,11(8bH,10H)-dione (**8f**)*

Crystallized from ethanol–chloroform 1:1, (v/v). Orange powder (82% yield), mp = 173–176 °C. IR [KBr, *v* (cm^{-1})]: 1706, 1582,

1490, 1377, 1263, 1169. ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): δ 3.69 (t, J = 8.0 Hz, 1H, 8-H), 3.92 (s, 3H, OMe), 3.87 (ad, J = 8.0 Hz, 1H, 18-H), 5.45 (d, J = 8.0, Hz, 1H, 7-H), 5.96 (s, 1H, 12-H), 6.26 (ad, J = 10.0 Hz, 1H, 6-H), 6.80 (d, J = 9.0 Hz, 1H, 13-H), 7.13 (ad, J = 8.5 Hz, 1H, 5-H), 7.20 (ad, J = 7.5 Hz, 2H, 2 × phenyl-CH), 7.25 [overlapped signal, 1H, (*m*)-OMe-phenyl-H], 7.36–7.43 (m, 3H, 3 × phenyl-CH), 7.32 (dd, J = 4.0, 9,0 Hz, 1H, 3-H), 7.51 (t, J = 8.0 Hz, 1H, (*m*)-OMe-phenyl-H), 7.82 (d, J = 9.0 Hz, 1H, 14-H), 7.84 (s, 1H, (*m*)-OMe-phenyl-H), 7.98 (d, J = 7.5 Hz, 1H, (*m*)-OMe-phenyl-H), 8.26 (ad, J = 9.0 Hz, 1H, 4-H), 8.66 (ad, J = 3.0 Hz, 1H, 2-H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 47.3 C-18, 48.1 C-8, 55.8 OMe, 61.1 C-7, 65.0 C-12, 112.8 CH-(*m*)-OMe-phenyl, 113.8 C-17, 117.2 C-13, 120.0 C-6, 121.7 C-5, 121.8 C-3, 122.0 CH-(*m*)-OMe-phenyl, 125.6 C-16, 126.5 2 × CH-Ph, 129.2 CH-Ph, 129.3 2 × CH-Ph, 129.4 C-4, 130.5 CH-(*m*)-OMe-phenyl, 130.8 CH-(*m*)-OMe-phenyl, 130.8 C-14, 131.5 Cq-Ph, 134.4 Cq-(*m*)-OMe-phenyl, 140.0 C-19, 143.1 C-15, 147.2 C-2, 160.4 C-OMe, 174.9 C-9, 176.5 C-11, 195.1 C-20 ppm. Anal. Calcd. for $C_{31}H_{23}N_3O_4$: C, 74.24; H, 4.62; N, 8.38. Found: C, 74.27, H, 4.52; N, 8.45.

*Dimethyl 8-(phenylcarbamoyl)-8,9-dihydropyrrolo[2,1-c][4,7]phenanthroline-7,9-dicarboxylate (**9e**)*

Purified by column chromatography: CH_2Cl_2: MeOH (99.6:0.4, v:v). Orange red powder (30% yield), mp = 166–168 °C. IR [KBr, ν (cm^{-1})]: 2951, 1751, 1721, 1678, 1615, 1597, 1549, 1211, 1186. ^{1}H NMR (500MHz, $CDCl_3$, 25 °C): δ 3.81 (s, 3H, OMe), 3.89 (s, 3H, OMe), 3.36 (d, J = 3.0 Hz, 1H, 8-H), 6.28 (dd, J = 3.0 Hz, 1H, 9-H), 7.09 (t, J = 7.5 Hz, 1H, Ph-H), 7.32 (t, J = 7.5 Hz, 2H, Ph-H), 7.41 (d, J = 9.0 Hz, 1H, 11-H), 7.52 (dd, J = 8.5, 4.0 Hz, 1H, 3-H), 7.60 (ad, J = 8.0 Hz, 3H, 6-H, Ph-H), 8.09 (d, J = 10.0 Hz, 1H, 5-H), 8.15 (d, J = 9.0 Hz, 1H, 12-H), 8.50 (d, J = 8.5 Hz, 1H, 4-H), 8.86 (dd, J = 4.0, 1.0 Hz, 1H, 2-H), 10.19 (s, 1H, NH). ^{13}C NMR (125 MHz, $CDCl_3$): δ 51.3 O<u>C</u>H$_3$, 51.4 C-8, 53.4 O<u>C</u>H$_3$, 62.2 C-9, 115.9 C-14, 117.5 C-11, 118.5 C-7, 119.7 2 × C<u>H</u>-Ph, 6-H, 122.8 C-3, 124.1 CH-Ph, 125.0 C-13, 129.1 2 × CH-Ph, 129.9 C-4, 131.3 C-5, 133.8 C-12, 137.5 C-16, 151.1 C-15, 144.3 C-17, 149.0 C-2, 168.32 CO, 170.1 CO. Anal. Calcd. for $C_{26}H_{21}N_3O5$: C, 68.56; H, 4.65; N, 9.23. Found: C, 68.50, H, 4.58; N, 8.28.

*9-(4-Nitrobenzoyl)-6a,7,8,9-tetrahydropyrrolo[2,1-c][4,7] phenanthroline-7-carbonitrile (**10b**)*

Crystallized from ethanol–chloroform 1:1, (v/v). Dark red powder (45% yield), mp = 265–268 °C. IR [KBr, ν (cm^{-1})]: 2911, 2176, 1708, 1630, 1499, 1472, 1344, 792. ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): δ 2.20–2.26 (m, 1H, 8a-H), 2.88–2.93 (m, 1H, 8b-H), 3.50 (at, J = 5.5 Hz,1H, 7-H), 5.31 (at, J = 2.5 Hz, 1H, 15-H), 5.57 (dd, J = 9.0, 6.5 Hz, 1H, 9-H), 5.82 (ad, J = 10.5 Hz, 1H, 6-H), 6.53 (d, J = 9.0 Hz, 1H, 11-H), 7.26 (overlapped signal, 1H, 5-H), 7.33 (dd, J = 4.0, 9.0 Hz, 1H, 3-H), 7.77 (d, J = 9.0 Hz, 1H, 12-H), 8.25 (ad, J = 9.0 Hz, 3H, 2 × phenyl-H, 4-H), 8.44 (d, J = 8.5 Hz, 2H, 2 × phenyl-H), 8.65 (ad, J = 3.0 Hz, 1H, 2-H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 32.3 C-8, 35.9 C-7, 61.9 C-9, 62.2 C-15, 112.0 C-14, 116.6 C-11, 117.9 C-6, 122.0 C-3, 119.1 CN, 123.6 C-5, 124.7 2 × CH-Ph, 125.8 C-13, 129.1 C-4, 129.9 2 × CH-Ph, 131.4 C-12, 138.3 Cq-Ph, 139.6 C-16, 143.2 C-17, 147.3 C-2, 151.2 Cq-Ph, 196.2 C-18 ppm. Anal. Calcd. for $C_{23}H_{16}N_4O_3$: C, 69.69; H, 4.07; N, 14.13. Found: C, 69.78, H, 4.03; N, 14.19.

*Methyl 7-cyano-8,9-dihydropyrrolo[2,1-c][4,7]phenanthroline-9-carboxylate (**11e**)*

Crystallized from ethanol–chloroform 1:1, (v/v). Dark red powder (45% yield), mp = 230–232 °C. IR [KBr, ν (cm^{-1})]: 2948, 2175, 1723, 1630, 1276, 833, 792. ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): δ 3.15 (dd, J = 16.0, 4.0 Hz, 1H, 8b-H), 3.48 (t, J = 15.0 Hz, 1H, 8a-H), 3.84 (s, 3H, OMe), 5.22 (dd, J = 13.0, 4.5 Hz, 1H, 9-H), 6.96 (d, J = 10.0 Hz, 1H, 6-H), 7.28 (d, J = 9.5 Hz, 1H, 11-H), 7.70 (d, J = 10.0 Hz, 1H, 5-H), 7.90 (dd, J = 8.5, 2.5 Hz, 1H, 3-H), 8.82 (ad, J = 4.0 Hz, 1H, 2-H), 8.89 (d, J = 9.0 Hz, 1H, 12-H), 8.95 (d, J = 8.5 Hz, 1H, 4-H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 33.3 C-8, 53.1 O<u>C</u>H$_3$, 61.2 C-9, 72.1 C-7, 119.7 C-6, 121.2 C-11, 114.7 C-14, 119.1 CN, 122.7 C-3, 125.4 C-12, 127.6 C-5, 126.4 C-13, 138.3 C-4, 135.0 C-17, 140.2 C-16, 140.4 C-2, 153.0 C-15, 169.3 CO. Anal. Calcd. for $C_{18}H_{13}N_3O_2$: C, 71.28; H, 4.32; N, 13.85. Found: C, 71.30, H, 4.29; N, 13.88.

*9-(3-Methoxybenzoyl)-8,9-dihydropyrrolo[2,1-c][4,7]phenanthroline-7-carbonitrile (**11f**)*

Crystallized from ethanol–chloroform 1:1, (v/v). Dark red powder (40% yield), mp = 221–224 °C. IR [KBr, ν (cm^{-1})]: 3072, 2916, 2168, 1689, 1633, 1268, 1136, 794. ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): δ 3.02 (dd, J = 15.0, 5.0 Hz, 1H, 8b-H), 3.63 (t, J = 14.0 Hz, 1H, 8a-H), 3.88 (s, 3H, OMe), 6.03 (dd, J = 14.0, 5.0 Hz, 1H, 9-H), 6.81 (d, J = 9.5 Hz, 1H, 11-H), 6.89 (d, J = 10.5 Hz, 1H, 5-H), 7.25 (overlapped signal, 1H, 3-H), 7.48–7.52 (m, 2H, 2 × phenyl-H), 7.54–7.58 (m, 2H, 2 × phenyl-H), 7.87 (d, J = 10.0 Hz, 1H, 6-H), 7.98 (d, J = 9.0 Hz, 1H, 12-H), 8.45 (d, J = 8.5 Hz, 1H, 4-H), 8.80 (dd, J = 4.0, 1.0 Hz, 1H, 2-H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 33.6 C-8, 55.7 O<u>C</u>H$_3$, 64.6 C-9, 66.9 C-7, 113.6 CH-Ph, 116.8 C-5, C-11, 115.1 C-14, 119.6 CN, 121.3 C-3, CH-Ph, 122.8 CH-Ph, 129.7 C-4, 129.9 C-6, 130.5 CH-Ph, 133.3 C-12, 133.9 Cq-Ph, 138.1 C-16, 125.3 C-13, 148.5 C-2, 155.1 C-15, 143.7 C-17, 160.5 Cq-Ph, 191.9 CO. Anal. Calcd. for $C_{24}H_{17}N_3O_2$: C, 75.97; H, 4.52; N, 11.08. Found: C, 76.02, H, 4.49; N, 11.13.

Microbiology

Antimycobacterial activities of the compounds were performed by Center of Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) at Southern Research Institute[36–38].

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 50 × 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. Data points obtained from a dose response growth inhibition assay are

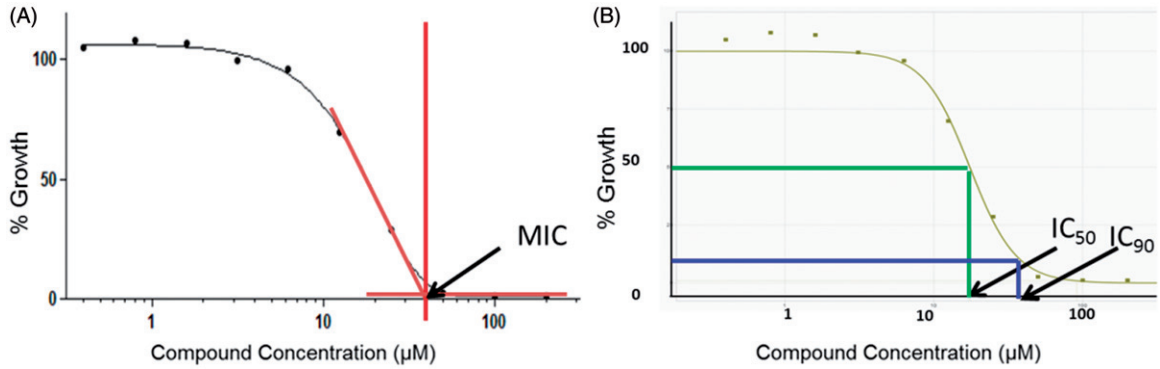


Figure 1. Dose response curves used to calculate MIC, IC_{50} and IC_{90}.

curve-fitted using the Gompertz model to calculate MIC (Figure 1A) and the Levenberg–Marquardt algorithm to calculate IC_{50} and IC_{90} (Figure 1B). (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. Orange line = MIC; Green line = IC_{50}; Blue line = 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. MICs were reported if
  - There were two points with growth >75%.
  - There were two 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

All monoquaternary 4,7-phenanthrolin-4-ium bromides **1** were prepared following our previously reported procedures[39]. Then, a variety of functionalized alkynes and alkenes were used as dipolarophiles for 3 + 2 cycloaddion of the *in situ* generated N-ylides **2** in order to synthesize a series of different substituted pyrrolo[2,1-*c*][4,7]phenanthroline derivatives. Thus, the dipolar cycloaddition reactions of the ylides **2(a–e)** [generated under triethylamine treatment from the corresponding salts **1**] to dimethylacetylene dicarboxylate (DMAD) and ethyl propiolate (EP) gave moderate yields of the expected aromatic pyrrolo[2,1-*c*][4,7]phenanthroline derivatives **4(a–e)** and **6(a–e)** (Scheme 1). From the reactions of ylides **2** with EP, only a single regioisomer has been isolated in accordance with the electronic effects in both reactants. The reaction of ylide **2a** and EP gave a mixture consisting of 8,9-dihydropyrrolo[2,1-*c*][4,7]phenanthroline derivative **7a** and aromatized compound **6a**. We assume that all cycloadditions in Scheme 1, result initially in formation of the unisolable 9,15-dihydropyrrolo[2,1-c][4,7]phenanthroline intermediates **3** and **5** that undergo an oxidative aromatization, converting into more stable aromatic compounds **4** and **6**. The formation of dihydroderivative **7a** takes place probably via a regioselective prototropic rearrangement.

The structures of the newly compounds were proven by elemental and spectral (IR, NMR, X-ray) methods.

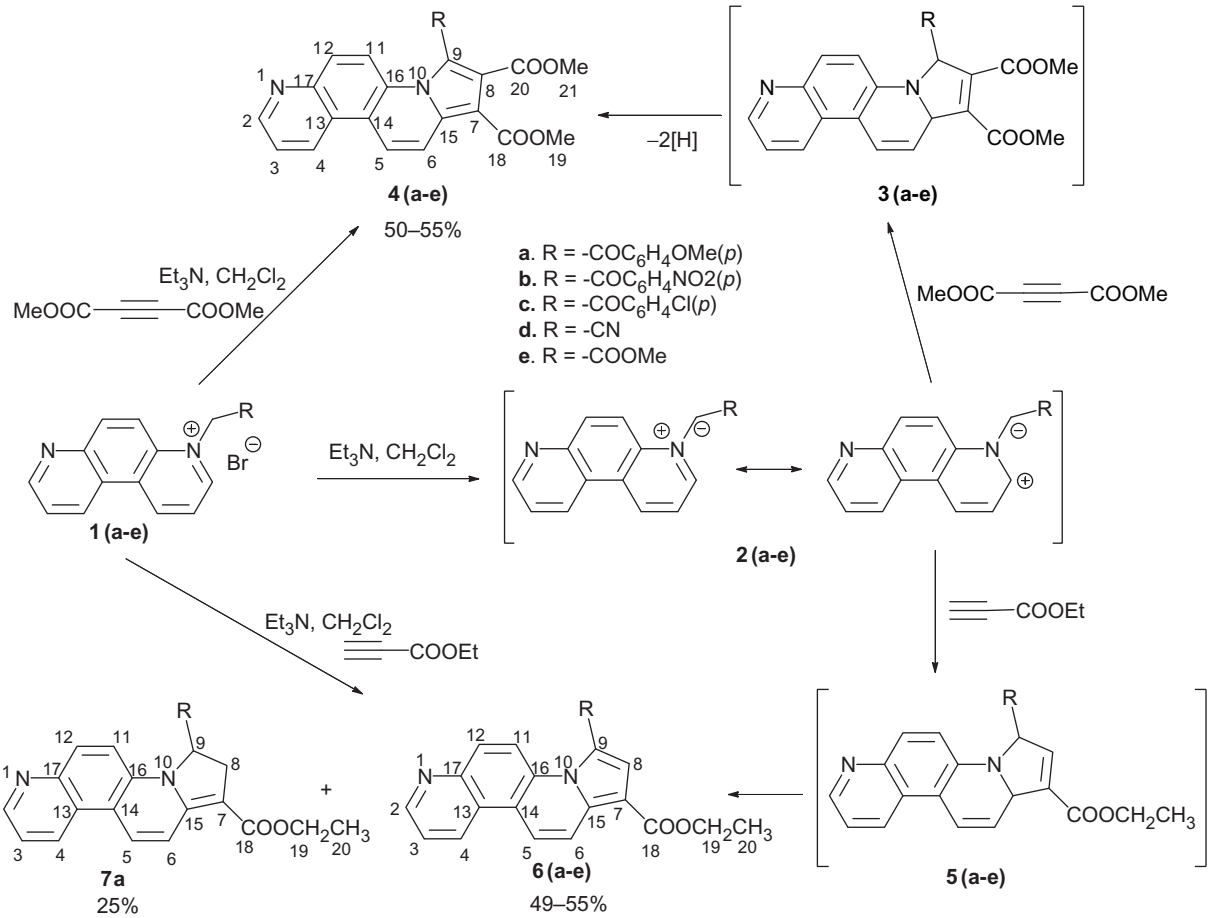
The ^{1}H and ^{13}C NMR spectra of compound **4** and **6** prove the pyrrolo ring formation. Interestingly, the signal of proton 11 that appear in the region of 7.87–8.54 ppm in the spectra of cycloadducts **4a–c**, **4e**, **6a–c** and **6e** shifts to the lower fields at 9.51 ppm in the spectrum of **4d**, and 9.46 ppm in the spectrum of **6d**, compounds in which the position 9 is substituted with a cyano group. We suppose that shifting is due to the positioning of the proton 11 in a deshielding anisotropy region of cyano group rather than a possible H-bond involving.

The position of the two protons on the dihydropyrrolo ring of **7a** were established on the basis of NMR and confirmed by single-crystal X-ray study. Thus, the ^{1}H-NMR spectrum of **7a** shows in the aliphatic region two signals at 3.09 and 3.64 ppm corresponding to the two geminal protons 8 coupled with the deshielded proton 9 that furnishes a signal at 6.99 ppm. As well, the chemical shift of the carbonyl ester groups shifts in ^{13}C-NMR spectrum shows an attachment to a C*sp2* (166.1 ppm). The result of single crystal X-ray study of compound **7a** (crystallized from CH_2Cl_2:MeOH 1:1 (v:v)) is shown in Figure 2, while the bond lengths and angles are summarized in Supplemental Table S2. The X-ray data show that dihydropyrrolo[2,1-*c*][4,7]phenanthroline core is almost planar, the maximal deviation of the atoms from the mean plane being 0.082 Å.

In the crystal structure, molecules **7a** are stocked through the CH···O hydrogen bonding into a columnar array aligned parallel to the *c* crystallographic axis, as shown in Figure 3. Significant π–π stacking interactions are also present within the column. The ring-centroid separation between inversion molecules are of 3.732 Å, and shift distance of 1.711 Å. The crystal structure of compound **7a** clearly demonstrated that it crystallizes without any solvent molecules.

Next step in our research plan was the study of 3 + 2 cycloaddition of ylides **2b**, **e**, **f** to symmetrical (N-phenylmaleimide) and unsymmetrical (acrylonitrile) substituted alkenes. Thus, N-ylides **2** were *in situ* generated and reacted with N-phenylmaleimide giving dihydropyrrolo[3′,4′:3,4]pyrrolo[2,1-*c*][4,7]phenanthroline derivatives **8** (Scheme 2).

The cycloaddition reactions with N-phenylmaleimide occur highly stereoselective, a single isomer (**8**) being obtained. Interestingly, from the reaction of **1e** in the same conditions, we isolated compound **9e** formed by hydrolysis of unstable intermediate **8** during column chromatography separation process. Thus, using CH_2Cl_2/MeOH (99.6:0.4; v/v) as eluent under acidic conditions on silica gel, the imidic cycle opened forming compound **9e**. As far, for the cycloaddition with acrylonitrile, the reactions are as expected highly regioselective. The reaction pathway with acrylonitrile occurs differently according the



Scheme 1. Synthesis of fused pyrrolo derivatives **4**, **6** and **7**.

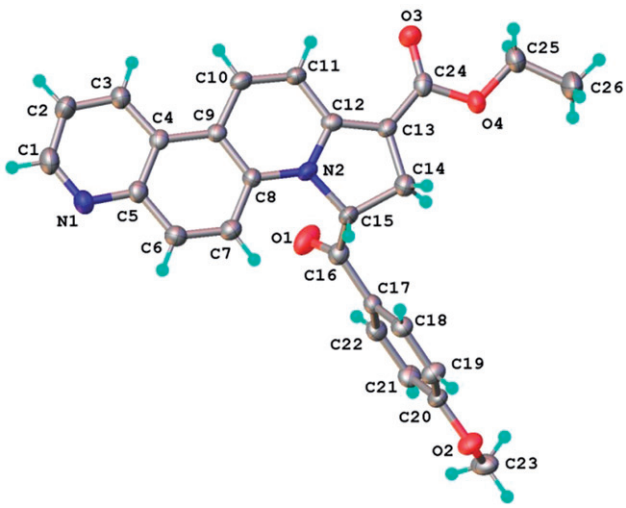


Figure 2. X-ray molecular structure of compound **7a** with thermal ellipsoids at 50% probability level.

starting ylide we used: a single compound with tetrahydropyrrolo structure **10b** was obtained in the case of ylide type **b**, while for ylides type **e** and **f**, a dihydroderivative (**11e** and **11f**, respectively) have been obtained. The formation of dihydroderivatives **11e** and **11f** could be explained by an oxidative dehydrogenation of tetrahydro intermediates **10**, leading to thermodynamically more stable product **11**.

In ^{1}H NMR of compounds **8**, there are four distinct signals in the aliphatic region corresponding to the four hydrogen atoms on the pyrrolo ring. The signals delivered by H12 appear as singlets, the lack of coupling for H12 proton being a proof for a *trans* position relative to proton 18. On the other hand, the big values for the coupling constants $J_{18,8}$ (8.0 Hz) and $J_{8,7}$ (8.0 Hz) show us that hydrogen atoms H18, H8 and H7 lay on the same side of the tetrahydropyrrolic ring, but on the opposite side of H12.

The structure of compound **9e** is proved by the existence in the ^{1}H NMR spectrum, of two singlets in the aliphatic region (3.81 and 3.89 ppm) corresponding to the methyl ester groups, two coupled doublets (at 4.36 and 6.28 ppm) corresponding to the dihydropyrrolo hydrogens and the singlet at low field (10.19 ppm) furnished by the amidic proton.

The presence of five signals in the aliphatic region of the ^{1}H NMR of compound **10b**, corresponds to the expected tetrahydropyrrolo ring hydrogen atoms, the most deshielded being H9 (dd, 5.57 ppm).

The similarity of ^{1}H NMR spectra of compound **11e**, **11f** and **7a** diminished the work for elucidation of structure **11**, these compounds being probably obtained by an oxidative process in which the intermediate compounds **10** lose two hydrogen atoms. The single crystal X-ray diffraction investigation has demonstrated that the structure of compound **11f** [crystallized from CH_2Cl_2:MeOH 1:1 (v:v)] is built up from molecular entities depicted in Figure 4, which confirmed the conclusions made on the base of above-mentioned methods.

The crystal structure of compound **11f** resembles that of compound **7a**, since the core is planar (the maximal deviation of the atoms from the mean plane is 0.079 Å) and similar columnar supramolecular architecture represents the main packing motif. The molecules **11f** are interacting within the column through the CH···O hydrogen-bonding donation to the oxygen atom O1 and

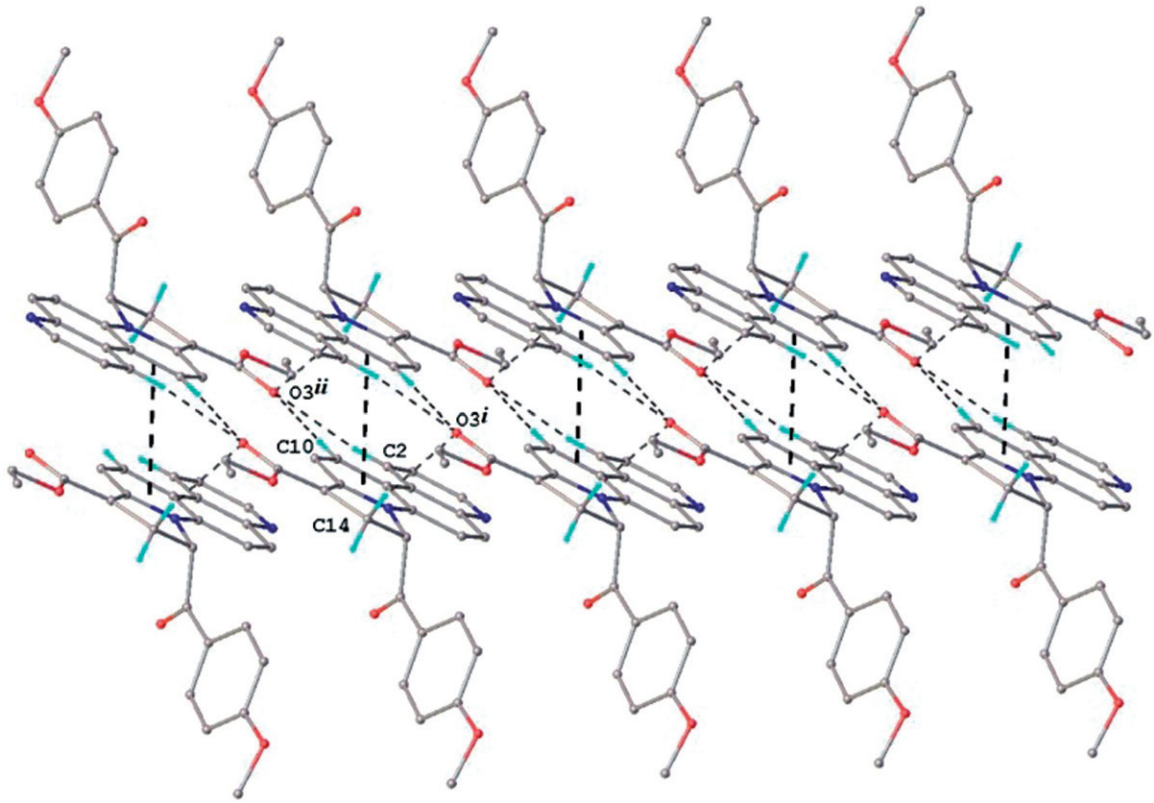
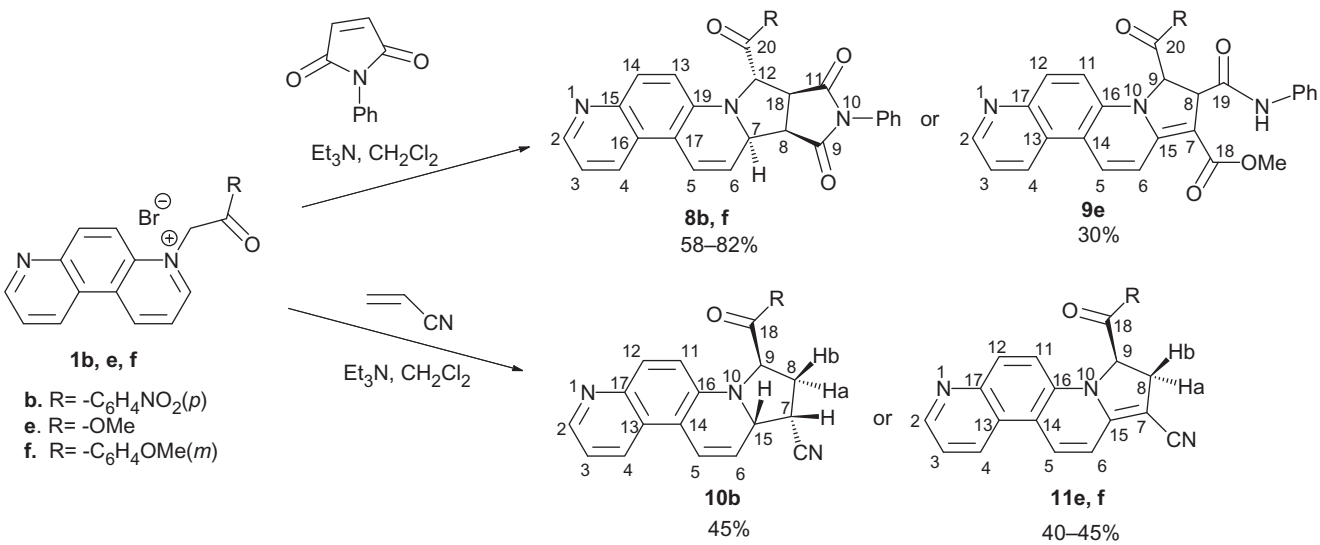


Figure 3. View of supramolecular columnar architecture in the crystal structure of **7a**. Only H atoms involved in hydrogen bonding are shown. H-bonds parameters: C10–H· · ·O3 [C10–H 0.93 Å, H· · ·O3 2.38 Å, C10· · ·O3(2 – *x*, 1 – *y*, –*z*) 3.308(4) Å, C10–H· · ·O3 175.8 °]; C3–H· · ·O3 [C3–H 0.93 Å, H· · ·O3 2.49 Å, C3· · ·O3(2 – *x*, 1 – *y*, –*z*) 3.419(4) Å, C10–H· · ·O3 173.0°]; C14–H· · ·O3 [C14–H 0.97 Å, H· · ·O3 2.44 Å, C14· · ·O3(–1 + *x*, *y*, *z*) 3.299(4) Å, C10–H· · ·O3 148.0°].



Scheme 2. Synthesis of fused pyrrolo derivatives **8**, **9**, **10** and **11**.

π–π stacking interactions, as shown in Figure 5. The ring-centroid distance between parallel packed aromatic systems exhibits the value of 3.757 Å and shift distance of 1.740 Å. No molecules of solvent are present in the crystal structure of compound **11f**.

Design and biological activity

We have recently discovered that 1-[2-(4-methoxyphenyl)-2-oxoethyl]-4-[[1-(ethoxycarbonyl)-3-benzoyl]-indolizin-7-yl]pyridinium bromide (Scheme 3) is a potent compound against both replicating and non-replicating *M. tuberculosis*[25]. On the other hand, phenanthroline, azaindolizine and phenanthridine derivatives were successful identified as DNA intercalating agents or possessing antimycobacterial activity[26,30,40–43]. In the same time, benzo[*c*]phenanthridinium alkaloids as fagaronine and nitidine which showed antileukemic activity on rodents[44] and act as topoisomerase I and II inhibitors[45–47], played the role of model compound for the development of new anticancer agents[14]. Having all these above consideration in mind and encouraged by our previous promising results in the field of anti-TB[25–28] and anticancer[29,30,32] derivatives with indolizine and/or phenanthroline skeleton, we have focused on the design of novel structures

that contain four or five fused (hetero)cycles with pyrrolo[2,1-*c*][4,7]phenanthroline skeleton (Scheme 3). In equal measure, we were interested to see the influence of the substituents at pyrrolo ring (especially the pharmacophoric moiety *p-chloro*-benzoyl[26]) on the pharmacological properties, and if the increasing of number of fused rings from four to five will affect these properties (Scheme 3).

The pyrrolo[2,1-*c*][4,7]phenanthroline derivatives **4** and **6** have been evaluated for *in vitro* antimycobacterial activity against *M. tuberculosis H37Rv* (grown under aerobic conditions), as a part of the TAACF TB screening program under direction of the US National Institute of Health, the NIAID division. Initially, the relative solubility of compounds in microbiological medium was measured using turbidity as a measure (Table 1). A serial dilution of compounds was prepared in DMSO and then transferred to microbiological medium at pH 6.8. Turbidity was measured and compared to a control; the lowest concentration at which compounds are insoluble (defined as turbidity > 300% of control) was recorded[48]. The data from Table 1 illustrate that all tested indolizine with phenanthroline skeleton have a good solubility in microbiological medium, which is quite promising from the pharmacological properties point of view. Then, the IC_{50}, IC_{90} and MIC of compound were determined by measuring bacterial growth after 5 days in the presence of test compounds[36–38,49]. The obtained results are listed in Table 1.

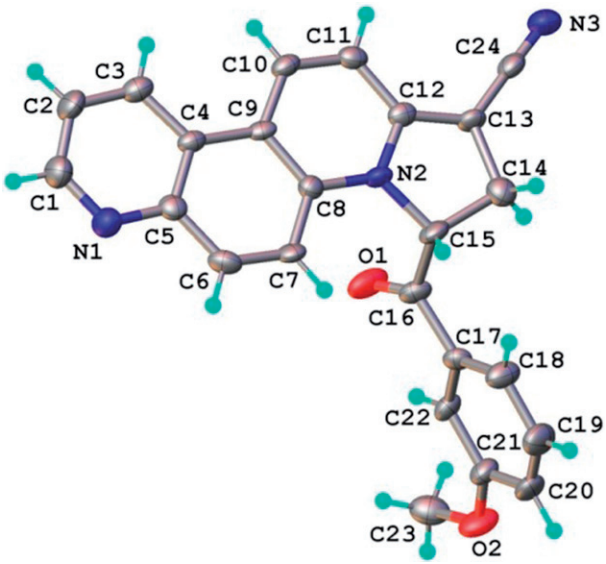


Figure 4. The X-ray molecular structure of compound **11f** with the thermal ellipsoids at 50% probability level.

As can be seen, five from the eight tested compounds (**4a–d** and **6a–d**) had activity against *M. tuberculosis H37Rv* under aerobic conditions, with an IC_{50} in a range of 20–50 μM. We may also notice that the presence of *p-chloro*-benzoyl moiety at position 9 did not affect the antimycobacterial activity of compounds **4c** and **6c** comparing with the other derivatives type **4** and **6**. Unfortunately, we are not able at the moment to comment the influence of the number of rings on the biological activity because the antimycobacterial evaluation of compounds **8**, **9**, **10** and **11** is underway.

Four indolizines with phenanthroline skeleton, **4a**, **4c**, **6c** and **6d**, were selected and tested *in vitro* for anticancer activity by the National Cancer Institute (NCI, USA), under the Developmental Therapeutics Program (DTP), at a single high dose (10^{-5} M) cell assay. This assay was performed in a 60 human tumor cell line panel, representing leukemia, melanoma and cancers of lung, colon, brain, breast, ovary, kidney and prostate, in accordance with the protocol of the NCI [US National Cancer Institute (NCI), Bethesda; http://dtp.nci.nih.gov/][50–52]. The results are expressed as ''percentage growth inhibition'' (PGI) term, and represent growth relative to the no-drug control, and relative to the time zero number of cells (Table 2). This allows detection of both growth inhibition (values between 0 and 100) and lethality (values less than 0)[51]. Supplementary information concerning the *in vitro* anticancer assay could be found in a research overview by Michael R. Boyd[52] and in the ESI of this article.

The results from Table 2 indicate that pyrrolo [2,1-*c*][4,7]phenanthroline derivatives **6c** and **6d** exhibit a significant antitumor growth inhibitory activity (around 50%) against renal cancer (UO-31) and breast cancer (MCF7), respectively. We may also notice a weaker antitumor growth inhibitory activity (around 25%) of **6c** against CNS Cancer (SF-SNB-19), **4a** against Melanoma (M14) and **6d** against Breast Cancer (T-47D).

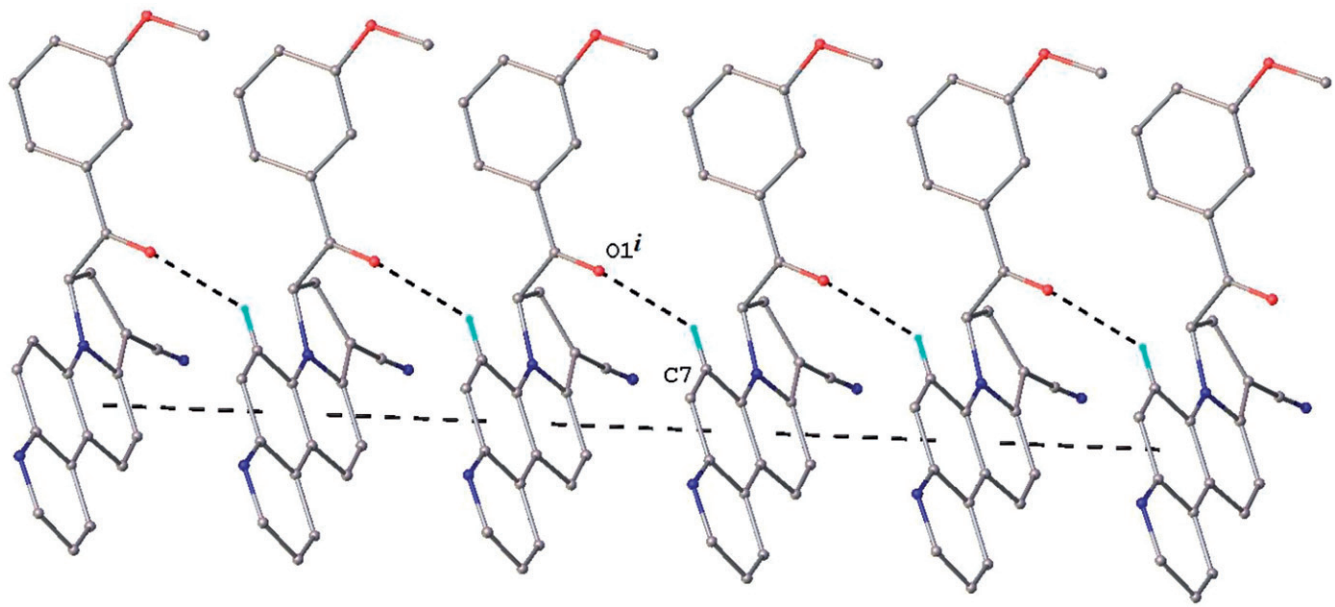
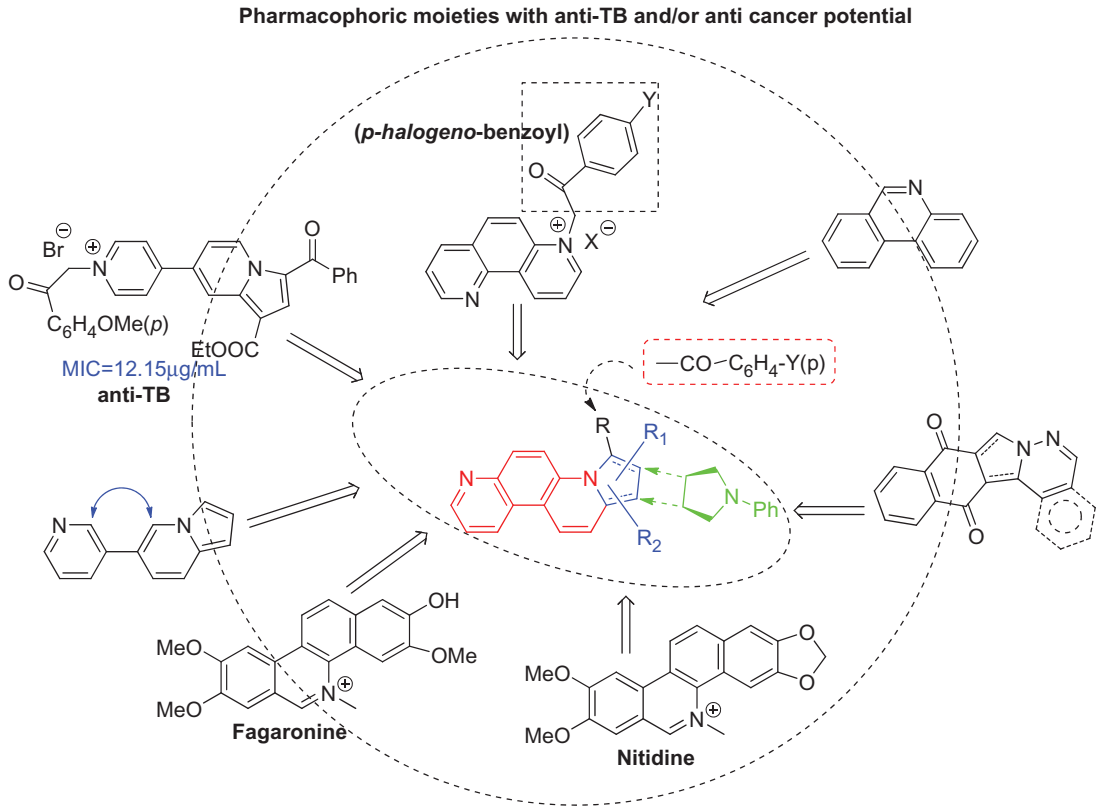


Figure 5. View of supramolecular columnar architecture in the crystal structure of **11f**. Only H atoms involved in hydrogen bonding are shown. H-bonds parameters: C7–H···O1 [C7–H 0.93 Å, H...O1 2.48 Å, C7···O1(1 + *x, y, z*) 3.158(5) Å, C10–H···O3 129.8 °].



Scheme 3. Design in the class of pyrrolo[2,1-*c*][4,7]phenanthroline derivatives.

Table 1. Solubility in microbiological medium and antimycobacterial activity of compounds **4a–d** and **6a–d** against *M. tuberculosis* H37Rv under aerobic conditions.

				Compound solubility	
Compounds	IC_{50} (μM)	IC_{90} (μM)	MIC (μM)	Starting concentration (μM)	Lowest insoluble concentration (μM)
4a	>200	>200	>200	200	12.5
4b	**>50**	**>50**	**>50**	100	12.5
4c	>100	>100	>100	20	10
4d	**>50**	**>50**	**>50**	50	12.5
6a	**>50**	**>50**	**>50**	20	20
6b	**>20**	**>20**	**>20**	200	N/A
6c	**>50**	**>50**	**>50**	200	100
6d	>200	>200	>200	200	N/A
Control*					
Rifampicin	0.0036	0.0061	0.0055	0.04	N/A

N/A means the compound was soluble at the highest concentration.
*Each experiment runs included and other control compounds: metoprolol tartrate, phenytoin, haloperidol, simvastatin, diethylstilbestrol and tamoxifen.
Bold values indicate moderate active compounds.

Table 2. Percentage growth inhibition (PGI, μM) data of compounds **4a**, **4c**, **6c** and **6d** against an NCI 60 human tumour cell lines (selection).

Panel/Cell line	Compound **4a**, PGI	Compound **4c**, PGI	Compound **6c**, PGI	Compound **6d**, PGI
CNS Cancer				
SF-SNB-19	84.77	95.43	74.19	86.66
Melanoma				
M14	77.14	88.38	95.18	99.07
Renal Cancer				
UO-31	81.49	84.21	**58.02**	76.07
Breast Cancer				
MCF7	84.73	92.59	93.10	**56.64**
T-47D	80.69	81.09	86.01	70.76

The number reported for the one-dose assay, percentage growth inhibition (PGI), is growth relative to the no-drug control and relative to the time zero number of cells. Full results are presented in the SM.
Bold and underlined values indicate active compounds. Underlined values indicates moderate active compounds.

As to the mechanism, taking into account, the above consideration concerning biological activity, as well as the fact that the X-ray structure of the new indolizines with phenanthroline skeleton show coplanar cores of these compounds, we assert that an interaction with DNA via an intercalation mechanism would be reasonable.

Conclusions

A new class of compounds with pyrrolo[2,1-*c*][4,7]phenanthroline skeleton has been synthesized by [3 + 2] dipolar cycloaddition of 4,7-phenanthrolin-4-ium ylides generated *in situ* from the corresponding monoquaternary salts, to activated symmetrical and unsymmetrical substituted alkynes or alkenes. The cycloaddition reactions to symmetrically substituted alkenes (*N*-phenylmaleimide) occur highly stereoselective (a single isomer being obtained), whereas the cycloadditions to

unsymmetrically substituted dipolarophiles (acrylonitrile and ethyl propiolate) occur highly regioselective (a single regioisomer being formed), under charge control. The formation of aromatized and dihydro-fused polycyclic indolizines (in some cases) was explained by an oxidative dehydrogenation of intermediate tetrahydro-fused derivatives, process that leads to thermodynamically more stable compounds. The structure of the new compounds was proved by elemental and spectral analysis, including X-ray diffraction. The antimycobacterial activity of eight compounds was investigated against *Mycobacterium tuberculosis H37Rv* under aerobic conditions, five of the tested compounds showing moderate activity. Four new compounds have been evaluated by NCI for anticancer activity. Two of the compounds exhibiting a significant antitumor growth inhibitory activity (around 50%) against renal cancer (UO-31) and breast cancer (MCF7), respectively.

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Declaration of interest

The authors report no conflict of interest. C. M. Al Matarneh is thankful for the financial support of grant POSDRU/159/1.5/S/137750, Project ''Doctoral and Postdoctoral programs support for increased competitiveness in Exact Sciences research'' co-financed by the European Social Found within the Sectorial Operational Program Human Resources Development 2007–2013.

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Supplementary material available online
Supplemental Tables S1 and S2.