Original Article

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Synthesis and bioevaluation of new pyranophenothiazine derivatives

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Abstract

Until now, tuberculosis is always a dangerous infectious disease. Because of the enlargement of multidrug-resistant strains (MDR-TB), the need of new drugs becomes more important. Fusing of 2 pharmacophores (phenothiazine and benzopyran), we intend to synthesize some new pyranophenothiazine derivatives, and evaluate their antituberculosis activities. The designed compounds were prepared using conventional synthetic methods with all PA chemical agents (from Merk, TCI, Sigma). The structure of all synthesized compounds had been confirmed by analysis of NMR and MS spectra. Antimycobacterial activity of the synthesized compounds on *Mycobacterium bovis BCG* (or on the virulent strain *Mycobacterium tuberculosis* H37Rv for the most promising compounds) had been determined using the Microdilution resazurin assay. 2 compounds 8 and 9 were obtained by Claisen rearrangement of intermediate dimethylpropargyl ether. The sulfoxide and (+)cis-diol sulfoxide derivatives were obtained by oxydation, and the dihydro-derivatives - by reduction of the leader compounds. The anti-tuberculosis activities of 12 synthesized compounds were evaluated. Only the leading compound of linear structure (pyrano*[b]*phenothiazine) and 2 non-sulfoxide derivatives of angular structure (pyrano*[a]*phenothiazine) exhibited a mild inhibiting activity on the tested tuberculosis source. 12 new pyrano-phenothiazine compounds have been prepared and bioevaluated. The results showed that 3 compounds exhibited a mild inhibiting activity on the tested tuberculosis source. These structural requirements will be taken into account for the design of further analogues in pyranophenothiazine series.

Keyword: pyranophenothiazine, chromene, antituberculosis, phenothiazine derivatives

1. INTRODUCTION

In 2015, tuberculosis caused estimated 10.4 million new cases worldwide, including about 480 000 new cases of multidrug-resistant TB (MDR-TB) and an additional 100 000 people with rifampicin-resistant TB (RR-TB), and responsed 1.8 million deaths[1]. So it remains one of the most dangerous infectious diseases in the world. The current chemotherapy established more than thirty years ago gives rise, since the 1990s, to multidrug-resistant strains (MDR-TB) particularly among the HIV-infected people where co-infection with *M. tuberculosis* is responsible of over 50 % of deaths. Consequently, the need of new drugs has never been greater[2, 3]. Intend to create new antituberculosis substances, we would like to hybridize 2 pharmacophores: phenothiazine and 2*H*-benzopyran (or 2*H*-chromene) because of their interesting bioactivities.

The phenothiazine (1) had been determined in 1883 by Heinrich August Bernthsen after

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the synthesis of blue methylene - an antiparasitic compound - in 1876 by Heinrich Caro. After the discovery of chlorpromazine (2) in 1952, more than 5000 phenothiazine derivatives were synthesized and evaluated biological activity, and many substances had been used clinically as anticonvulsant, antipsychotic, antiemetic, antimicrobial, antihistamine, antitumor agent[4]. Several phenothiazine derivatives such as chlorpromazine, thioridazine (3) had showed antituberculosis activity which able to circumvent the MDR resistance[5-8]. So this tricyclic basic core had been chosen as a part of new antituberculosis structure.

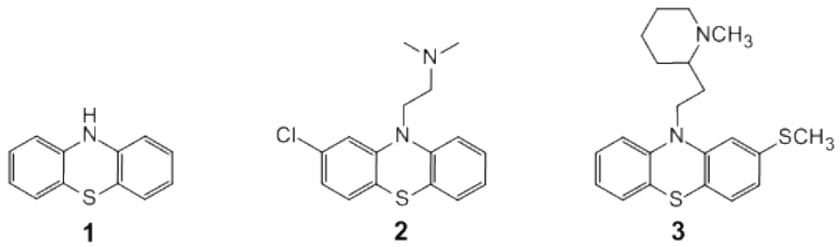


Figure 1. Structures of phenothiazine (1), chlorpromazine (2) and thioridazine (3)

The 2*H*-benzopyran appeared as a "privileged substructure" present in numerous bioactive compounds[9] exemplified by the antifungal benzopyran methylripariochromene A (4)[10], the antibacterial pyranoflavanone 5-methyllupinifoliol (5)[11], and the antitumor acridone alkaloid acronycine (6)[12, 13]. In the course of our research, the 3,3-dimethyl-3H-benzofuro[3,2-*f*][1]-benzopyran (7) had been synthesized and demonstrated promising and specific antituberculosis properties[14, 15]. So the 2*H*-benzopyran may give an interesting activity for the new structure.

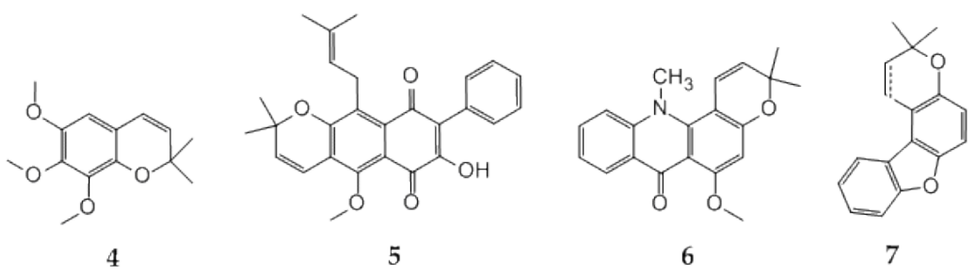


Figure 2. 2*H*-benzopyran as a privileged substructure

The purpose of this research was to synthesize potential antituberculosis compounds, including a phenothiazine basic core fused with an additional pyran ring, leading to two different series depending of the linear (8) or angular (9) mode of fusion. The double-bond of the fused dimethylpyran ring would be modified, permitting modulation of molecular lipophilicity in the course of structure activity relationship studies.

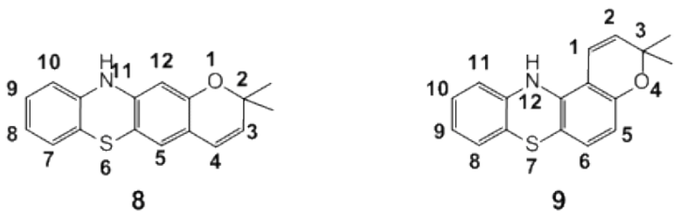


Figure 3. Structures of the two series of pyranophenothiazines

2. MATERIALS AND METHODS

2.1. Materials

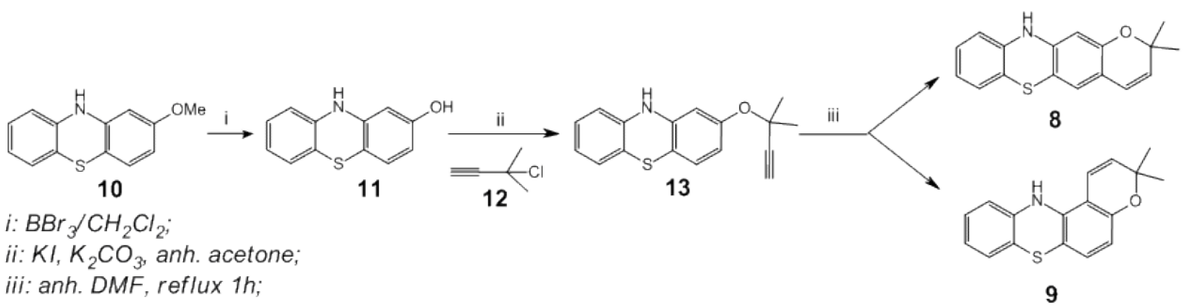
The designed compounds were prepared using conventional synthetic methods with all PA chemical agents (from Merck, TCI, Sigma). IR spectra were recorded on a Nicolet 510 FT-IR spectrophotometer as KBr discs. ^{1}H and ^{13}C NMR spectra were recorded on an AC 300 Bruker spectrometer (300 MHz for ^{1}H and 75 MHz for ^{13}C) and an Avance 400 Bruker spectrometer. Mass spectra were recorded on a Nermag R 10-10C mass spectrometer (DIC/NH_3) (90eV), or a Hewlett Packard 5890 spectrometer (IE) (70eV), or a ZQ 2000 Waters spectrometer (ESI).

2.2. Methods

2.2.1. Chemistry

The synthesis of desired compounds was realized by 2 steps: 1) synthesis of compounds 8 and 9, and 2) synthesis of their derivatives.

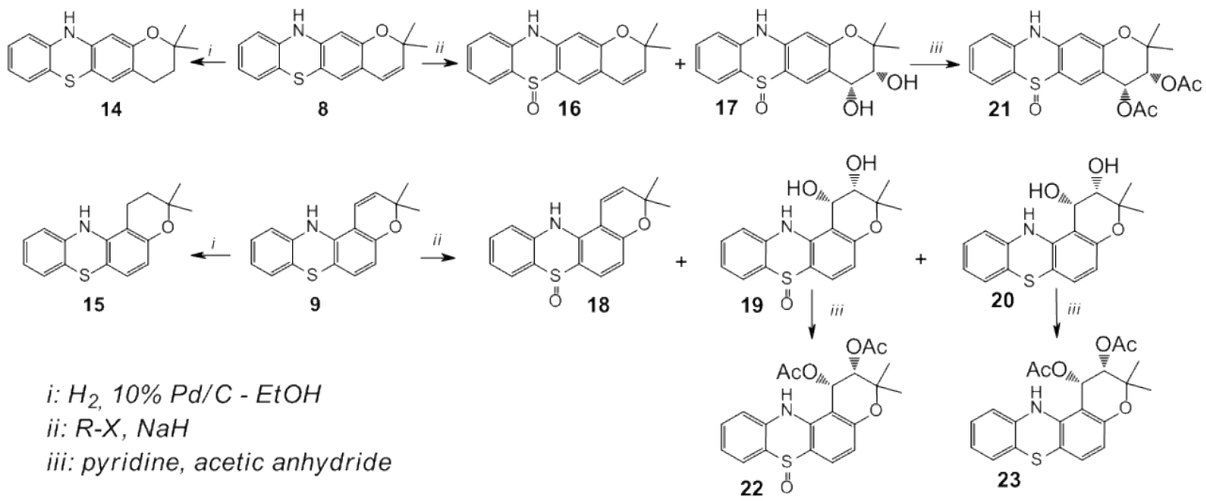
In the first step, 2 compounds 8 and 9 were synthesized according to the pathway depicted in scheme 1.



Scheme 1. Synthesis of 2 leading compounds

A demethylation of 2-methoxyphenothiazine (10) had given 10*H*-2-hydroxyphenothiazine (11), which was put into the reaction with 3-chloro-3-methylbut-1-yne (12)[16] to form 2-(1,1-dimethyl-propargyloxy)-phenothiazine (13) in overall yield of about 50%. This propargylic ether had been cyclized intramolecularly at high temperature to afford the expected products 8 and 9 at the proportion of 2:1 respectively.

The derivatives of these compounds (8 and 9) were synthesized in the second step according to the pathway depicted in scheme 2.



Scheme 2. Synthesis of pyranophenothiazine derivatives

The hydrogenation of compound 8 or 9 gave reduced product 14 or 15 respectively. In other direction, the oxidation of compound 8 gave the sulfoxyde compound (without hydroxylation) 16 and (+)*cis*-dihydroxysulfoxyde derivatives 17. The oxidation of 9 gave not only 2 products at different levels of oxidation as happened in linear serie (the sulfoxyde without hydroxylation 18 and the (+)*cis*-dihydroxysulfoxyde 19), but also the *cis*-dihydroxy derivative without sulfoxyde 20.

Each (+)*cis*-dihydroxy product (17, 19, 20) was treated with excess acetic anhydride in pyridine to give the corresponding diacetate (21, 22, 23).

The structure of all synthesized compounds had been confirmed by analysis of NMR and MS spectra.

2.2.2. Bioactivity

Antimycobacterial activity of the synthesized compounds on *Mycobacterium bovis* BCG (or on the virulent strain *Mycobacterium tuberculosis* H37Rv for the most promising compounds) was determined using the Microdilution resazurin assay (MRA)[17]. Resazurin salt powder (Sigma) was prepared at 0.01% (wt/vol) in distilled water, sterilized by filtration through a 0.22 µm membrane and stored at 4°C for a week. Drug stock solutions were prepared in dimethylsulfoxide (DMSO) at concentration of 50 mg/mL and frozen until used. The inocula were prepared from *M. tuberculosis* H37Rv and *M. bovis* BCG strains grown in Dubos medium supplemented with 10% ADC enrichment (Difco). 2 µL of two fold serial dilutions of each drug was prepared in 200 µL of Dubos medium directly in 96-well plates at concentrations from 100 to 0.05µg/mL. Growth controls containing DMSO and isoniazide (from 1 µg/mL to 1 ng/mL) were also included. The plates were covered, sealed and incubated at 37 °C. After 6 days for *M. tuberculosis* or 8 days for *M. bovis*, 30 µL of resazurin solution was added to each well and plates were allowed to incubate at 37°C for an additional 24 h. A change from blue to pink indicated reduction of resazurin and therefore bacterial growth. The MIC was defined as the lowest drug concentration that prevented this color change.

3. RESULTS

3.1. Chemistry

12 pyranophenothiazines had been synthesized. The information of their spectra of ^{1}H - NMR, ^{13}C- NMR, MS, and IR was given below.

10*H*-2-Hydroxyphenothiazine (11) : amorphous white product: UV (MeOH) λ_{max}:

213.7; 253.3; 319.2 nm; IR (KBr) $\sqrt{}_{max}$ (cm^{-1}): 3338, 3280, 1600, 1575, 1452, 1397, 1230, 1203, 1179, 855, 751; ^{1}H- NMR (DMSO-d6) δ(ppm): 9.29 (1H, s, OH); 8.47 (1H, s, NH); 6.98 (1H, dt, J=8, 1 Hz, H8); 6.88 (1H, dd, *J*=8, 1 Hz, H_6); 6.72 (1H, dt, *J*=8, 1 Hz, H_7); 6.67 (2H, m, H_9, H_4); 6.22 (1H, br s, H_1); 6.20 (1H, dd, *J*=8, 2 Hz, H_3); ^{13}C- NMR (DMSO-d_6) δ(ppm): 157.7 (C_2); 143.8 (C_{10a}); 142.5 (C_{9a}); 127.7 (C_8); 127.3 (C_4); 126.6 (C_6); 122.0 (C_7); 117.8 (C_{5a}); 114.8 (C_9); 109.5 (C_3); 105.4 (C_{4a}); 102.6 (C_1); $C_{12}H_9NOS$ (215); Mass (DIC/NH_3) *m/z*: 216^{11+}, 233 $[M+NH_4]^+$;

10*H*-2-(1,1-Dimethyl-prop-2-ynyloxy)-phenothiazine (13) : amorphous yellow product (423 mg – 65% yield): IR: (KBr) $\sqrt{}_{max}$ (cm^{-1}): 3354, 3259, 2986, 1600, 1585, 1571, 1459, 1433, 1299, 1265, 1223, 1163, 1143, 1124, 982, 878, 856, 750, 681; ^{1}H - NMR (DMSO-d_6), δ (ppm): 8.60 (1H, br s, NH); 6.97 (1H, t, *J*=8 Hz, H_8); 6.90 (1H, d, *J*=8 Hz, H_6); 6.81 (1H, d, *J*=9 Hz, H_4); 6.75 (1H, m, H_7); 6.68 (1H, d, *J*=8 Hz, H_9); 6.57 (2H, m, H_1, H_3); 3.65 (1H, s, H_3'); 1.55 (6H, s, $2CH_3$); ^{13}C- NMR: 155.5 (C_2), 143.4 (C_{10a}), 142.4 (C_{9a}), 128.0 (C_8), 126.9 (C_4), 126.8 (C_6), 122.4 (C_7), 117.2 (C_{5a}), 115.0 (C_3, C_9), 110.4 (C_{4a}), 108.1 (C_1), 86.3 ($C_{2'}$), 77.4 ($C_{3'}$), 72.6 ($C_{1'}$), 29.9 (2 CH_3); $C_{17}H_{15}NOS$; Mass: (DIC/NH_3) m/z: 282 $([MH]^+)$;

11*H*-2,2-dimethyl-2*H*-pyrano[2,3-b] phenothiazine (8): amorphous yellow products IR (KBr) $\sqrt{}_{max}$ (cm^{-1}): 3331, 2973, 1611, 1581, 1473, 1357, 1306, 1259, 1158, 848, 735; ^{1}H-NMR (DMSO-d_6) δ(ppm) : 8.60 (1H, s, NH); 6.98 (1H, dt, J=8, 2 Hz, H_8); 6.90 (1H, dd, *J*=8, 2 Hz, H_7); 6.73 (1H, dt, *J*=8, 2 Hz, H_9); 6.65 (1H, dd, *J*=8, 2 Hz, H_{10}); 6.61 (1H, s, H_5); 6.22 (1H, d, *J*=10 Hz, H_4); 6.12 (1H, s, H_{12}); 5.52 (1H, d, *J*=10 Hz, H_3); 1.30 (6H, s, $2CH_3$);^{13}C-NMR: 153.1 (C_{12a}), 143.4 (C_{11a}), 142.0 (C_{10a}), 128.4 (C_3), 128.0 (C_8), 126.8 (C_7), 124.2 (C_5), 122.4 (C_9), 121.6 (C_4), 117.4 (C_{6a}), 116.2 (C_{4a}), 115.0 (C_{10}), 107.4 (C_{5a}), 103.0 (C_{12}), 76.7 (C_2), 28.1 (2 CH_3); $C_{17}H_{15}NOS$; Mass: (DIC/NH_3) m/z: $282^{11+;}$

12*H*-3,3-dimethyl-3*H*-pyrano[3,2-a] phenothiazine (9): IR: (KBr) $\sqrt{}_{max}$ (cm^{-1}): 3398, 2924, 1601, 1561, 1450, 1437, 1411, 1277, 1254, 1198, 1126, 1051, 809, 751, 712; ^{1}H- NMR (DMSO-d_6) δ(ppm): 8.20 (1H, s, NH); 7.02 (2H, dl, $H_{8,}$ H_{11}); 6.93 (2H, dt, *J*=8, 10 Hz, $H_{10,}$ H_1); 6.80 (1H, m, H_9); 6,71 (1H, d, *J*=8 Hz, H_6); 6.30 (1H, d, *J*=8 Hz, H_5); 5.81 (1H, d, *J*=10 Hz, H_2); 1.30 (6H, s, $2CH_3$); ^{13}C- NMR : 152.7 (C_{4a}), 142.3 (C_{11a}), 138.5 (C_{12a}), 130.8 (C_2), 12.8 (C_8), 126.9 (C_6), 126.6 (C_{10}), 122.8 (C_9), 118.5 (C_{7a}), 117.1 (C_1), 116.1 (C_{11}), 110.4 (C_5), 108.9 (C_{6a}), 108.5 (C_{12b}), 75.5 (C_3), 27.6 (2 CH_3); $C_{17}H_{15}NOS$; Mass: (DIC/NH_3) m/z: 282;

11*H*-3,4-Dihydro-2,2-dimethyl-2*H*-pyrano[2,3-b]phenothiazine (14)

The reduction of pyranophenothiazine 8 gave the corresponding compound 14 (66.3 mg – 94% yield) as amorphous yellowish powder: IR: (KBr) $\sqrt{}_{max}$ (cm^{-1}): 3329, 2968, 2926, 1614, 1596, 1583, 1473, 1433, 1379, 1303, 1166, 1123, 843, 735; ^{1}H- NMR (DMSO-d_6) δ(ppm): 8.45 (1H, s, NH); 6.97 (1H, dt, *J*=8, 2Hz, H_8); 6.89 (1H, d, *J*=8 Hz, H_7); 6.72 (1H, t, *J*=8 Hz, H_9); 6.65 (1H, d, *J*=8 Hz, H_{10}); 6.62 (1H, s, H_5); 6.09 (1H, s, H_{12}); 2.55 (2H, t, *J*=1 Hz, $2H_4$); 1.67 (2H, t, *J*=1 Hz, $2H_3$); 1.22 (6H, s, 2 CH_3); ^{13}C-NMR : 153.6 (C_{12a}), 142.5 (C_{11a}), 141.7 (C_{10a}), 127.7 (C_9), 126.9 (C_5), 126.6 (C_7), 121.8 (C_8), 117.5 (C_{6a}), 114.9 (C_{4a}), 114.7 (C_{10}), 107.0 (C_{5a}), 103.2 (C_{12}), 74.6 (C_2), 32.6 (C_4), 26.9 (2 CH_3), 21.5 (C_3); $C_{17}H_{17}NOS$; Mass: (TOF MS ES^+) m/z: 283 $([M]^+)$;

12*H*-1,2-Dihydro-3,3-dimethyl-3*H*-pyrano[3,2-a]phenothiazine (15)

The reduction of pyranophenothiazine 9 gave the corresponding compound 15 (67.0 mg – 95% yield) as an amorphous yellowish powder: IR: (KBr) $\sqrt{}_{max}$ (cm^{-1}): 3385, 2924, 1599, 1569, 1561, 1454, 1433, 1272, 1170, 1126, 1051, 806, 749, 634; ^{1}H- NMR (DMSO-d_6) δ(ppm): 7.68 (1H, s, NH); 7.02 (2H, m, $H_{8,}$ H_{11}); 6.92 (1H, d, *J*=8 Hz, H_{10}); 6.78 (1H, t, *J*=8 Hz, H_9); 6.66 (1H, d, *J*=8 Hz, H_6); 6.22 (1H, d, *J*=8 Hz, H_5); 2.57 (1H, t, *J*=6 Hz, H_1); 1.77 (1H, t, *J*=6 Hz, H_2); 1.25 (6H, s, $2CH_3$); ^{13}C- NMR: 153.7 (C_{4a}), 142.5 (C_{11a}), 141.3 (C_{12a}), 127.6 (C_8), 126.4 (C_6), 125.0 (C_{10}), 122.6 (C_9), 118.5 (C_{7a}), 116.2 (C_{11}), 111.0 (C_5), 107.9 (C_{6a}), 106.3 (C_{12b}), 73.7 (C_3), 31.8 (C_1), 26.6 ($2CH_3$), 18.6 (C_2); $C_{17}H_{17}NOS$; Mass: (TOF MS ES^+) m/z: 283 $([M]^+)$;

The oxydation of pyranophenothiazine 8 gave diol sulfoxide 16 (76.5 mg – 65% yield) and the sulfoxide 18 (15.5 mg - 14% yield) as amorphous white powders.

11*H*-2,2-Dimethyl-2*H*-pyrano[2,3-b] phenothiazine S-oxide (16): IR: (KBr) $\sqrt{}_{max}$ (cm^{-1}): 3448, 3246, 2976, 1637, 1619, 1592, 1509, 1476, 1179, 1162, 1111, 1060, 978; ^{1}H-NMR (DMSO-d_6) δ(ppm): 10.80 (1H, s, NH); 7.83 (1H, d, *J*=9Hz, H_7); 7.61 (1H, s, H_5); 7.56 (1H, dt, *J*=9, 1Hz, H_9); 7.30 (1H, dd, J=9, 1Hz, H_{10}); 7.15 (1H, dt, *J*=9, 1Hz, H_8); 6.65 (1H, s, H_{12}); 6.50 (1H, d, *J*=10Hz, H_4); 5.75 (1H, d, *J*=10Hz, H_3); 1.40 (6H, d, $2CH_3$); ^{13}C- NMR : 157.2 (C_{12a}), 139.2 (C_{11a}), 137.5 (C_{10a}), 133.5 (C_9), 132.1 (C_7), 130.6 (C_3), 130.1 (C_5), 122.3 (C_8), 121.8 (C_4), 117.8 (C_{10}), 117.2 (C_{4a}), 115.4 (C_{5a}), 103.4 (C_{12}), 78.4 (C_2), 29.1, 29.0 (2 CH_3); $C_{17}H_{15}NO_2S$; Mass: (DIC/NH_3) m/z: 298 ($[MH]^+$);

(±)-*cis*-11*H*-3,4-Dihydroxy-2,2-dimethyl-2*H*-pyrano[2,3-*b*]phenothiazine *S*-oxide (17): IR: (KBr) $\sqrt{}_{max}$ (cm^{-1}): 3504-3341, 1629, 1615, 1578, 1527, 1488, 1295, 1246, 1222, 1193, 1175, 1131, 1081, 1064, 1052, 845, 743; ^{1}H- NMR (DMSO-d_6) δ(ppm): 10.60 (1H, s, NH); 7.93 (1H, s, H_5); 7.85 (1H, d, *J*=8 Hz, H_7); 7.60 (1H, t, *J*=8; 1 Hz, H_9); 7.25 (1H, d, *J*=8 Hz, H_{10}); 7.18 (1H, t, *J*=8; 1 Hz, H_8); 6.50 (1H, s, H_{12}); 5.50 (1H, d, *J*=1 Hz, OH-4); 5.03 (1H, d, *J*=1 Hz, OH-3); 4.75 (1H, m, H_4); 3.60 (1H, m, H_3); 1.40 (3H, s, CH_3); 1.23 (3H, s, CH_3); ^{13}C- NMR : 157.3 (C_{12a}), 139.3 (C_{11a}), 139.0 (C_{10a}), 133.6 (C_9), 123.7 (C_5), 122.9 (C_7), 121.7 (C_8), 121.6 (C_{6a}), 120.7 (C_{4a}), 117.3 (C_{10}), 114.4 (C_{5a}), 101.8 (C_{12}), 80.2 (C_3), 70.4 (C_4), 64.1(C_2), 25.4, 25.0 (2 CH_3); $C_{17}H_{17}NO_4S$; Mass: (DIC/NH_3) m/z: 331 ($[M]^+$);

The oxydation of pyranophenothiazine 9 gave a mixture of 12*H*-3,3-dimethyl-3*H*-pyrano[3,2-*a*]phenothiazine *S*-oxide (18) (12 mg – 11% yield), (±)-*cis*-12*H*-1,2-dihydroxy-3,3-dimethyl-3*H*-pyrano[3,2-*a*]phenothiazine *S*-oxide (19) (60.8 mg – 70% yield) as amorphous white powders and (±)-*cis*-12*H*-1,2-dihydroxy-3,3-dimethyl-3*H*-pyrano[3,2-*a*]phenothiazine (20) (8 mg – 7% yield) an amorphous brown powder.

12*H*-3,3-Dimethyl-3*H*-pyrano[3,2-*a*] phenothiazine *S*-oxide (18): IR: (KBr) $\sqrt{}_{max}$ (cm^{-1}): 3277, 2973, 1612, 1578, 1570, 1455, 1285, 1264, 1123, 1066, 1006, 751, 723; ^{1}H-NMR (DMSO-d_6) δ(ppm): 10.15 (1H, s, NH); 7.86 (1H, d, *J*=8 Hz, H_8); 7.68 (2H, d, *J*=8 Hz, H_{11}, H_6); 7.60 (1H, t, *J*=8 Hz, H_{10}); 7.20 (1H, t, *J*=8 Hz, H_9); 7.18 (1H, d, *J*=8 Hz, H_1); 6.67 (1H, d, *J*=8 Hz, H_5); 5.94 (1H, d, *J*=8 Hz, H_2); 1.43 (6H, s, 2 CH_3); ^{13} C- NMR: 156.3 (C_{4a}), 137.2 (C_{11a}), 133.6 (C_{12a}), 132.9 (C_{10}, C_6), 131.2 (C_8), 130.6 (C_2), 122.3 (C_9), 122.1 (C_{7a}), 118.2 (C_{11}), 116.6 (C_1), 115.5 (C_{6a}), 111.4 (C_5), 108.1 (C_{12b}), 76.8 (C_3), 28.0, 27.6 (2 **C**H_3); $C_{17}H_{15}NO_2S$; Mass: (DIC-NH_3) *m/z*: 298 ($[MH]^+$);

(±)-*cis*-12*H*-1,2-Dihydroxy-3,3-dimethyl-3*H*-pyrano[3,2-*a*]phenothiazine *S*-oxide (19): IR: (KBr) $\sqrt{}_{max}$ (cm^{-1}): 3412, 3336, 1610, 1578, 1521, 1486, 1460, 1444, 1276, 1154, 1110, 1064, 1043, 761; ^{1}H- NMR (DMSO-d_6) δ(ppm): 9.90 (1H, s, NH); 7.90 (1H, d, *J*=8Hz, H_8); 7.71 (1H, d, *J*=9Hz, H_6); 7.64 (1H, t, *J*=8; 1Hz, H_{10}); 7.49 (1H, d, *J*=8Hz, H_{11}); 7.26 (1H, t, *J*=8; 1Hz, H_9); 6.66 (1H, d, *J*=9Hz, H_5); 5.69 (1H, dl, OH_1); 5.48 (1H, d, *J*=6Hz, OH_2); 5.07 (1H, m, H_1); 3.68 (1H, dd, *J*=4; 6Hz, H_2); 1.35 (3H, s, CH_3); 1.25 (3H, s, CH_3); ^{13}C- NMR : 156.6 (C_{4a}), 139.9 (C_{11a}), 138.4 (C_{12a}), 133.4 (C_{10}), 123.5 (C_6), 122.5 (C_8), 122.3 (C_9), 121.7 (C_{7a}), 118.5 (C_{11}), 114.0 (C_{6a}), 112.1 (C_5), 109.2 (C_{12b}), 79.3 (C_3), 70.5 (C_2), 63.1(C_1), 25.0, 23.5 (2 **C**H_3); $C_{17}H_{17}NO_4S$; Mass: (ES^+) *m/z*: 370 ($[MK]^+$);

(±)-*cis*-12*H*-1,2-Dihydroxy-3,3-dimethyl-3*H*-pyrano[3,2-*a*]phenothiazine (20): IR: (KBr) $\sqrt{}_{max}$ (cm^{-1}): 3367, 1607, 1570, 1453, 1434, 1267, 1214, 1146, 1100, 1039, 747; ^{1}H -NMR: (DMSO-d_6), δ(ppm): 8.05 (1H, s, NH); 7.00 (2H, m, $H_{8;}$ H_{10}); 6.80 (3H, m, H_6; H_9; H_{11}); 6.24 (1H, d, *J*=8 Hz, H_5); 5.42 (1H, br s, OH_1); 5.18 (1H, br s, OH_2); 4.80 (1H, br s, H_1); 3.55 (1H, br s, H_2); 1.28 (3H, s, CH_3); 1.22 (3H, s, CH_3); ^{13}C- NMR : 153.0 (C_{4a}), 143.4 (C_{12a}), 142.2 (C_{11a}), 127.8 (C_{10}), 126.8 (C_6), 126.6 (C_8), 122.8 (C_9), 118.3 (C_{7a}), 115.9 (C_{11}), 110.7 (C_5), 110.2 (C_{12b}), 107.1 (C_{6a}), 77.7 (C_3), 71.2 (C_2), 63.0(C_1), 25.4, 22.8 (2 CH_3);

$C_{17}H_{17}NO_3S$; Mass: (ES^+) *m/z*: 338 ([MNa]$^+$); The acetylation 30 mg of each compound 17, 19, 20 gave diacetate compound corresponding 21, 22, 23 as amorphous white powders with a yields of about 90%.

(±)-*cis*-12*H*-1,2-Diacetate-3,3-dimethyl-3*H*-pyrano[3,2-*b*]phenothiazine S-oxyd (21): IR: (KBr) $\sqrt{}_{max}$ (cm^{-1}): 1750 ; 1630 ; 1484 ; 1376; 1239; 1150; 1131; 1074; 1052; ^{1}H -NMR: (DMSO-d_6), δ(ppm): 10,90 (1H, s, NH); 7,90 (1H, d, *J*=8 Hz, H_7); 7,80 (1H, s, H_5); 7,60 (1H, t, *J*=8; 1 Hz, H_9); 7,27 (1H, d, *J*=8 Hz, H_{10}); 7,20 (1H, t, *J*=8 ; 1 Hz, H_8); 6,65 (1H, s, H_{12}); 6,15 (1H, d, *J*=5 Hz, H_4); 5,32 (1H, d, *J*=5 Hz, H_3); 2,10 (3H, s, 4-$OCOCH_3$); 2,03 (3H, s, 3-$OCOCH_3$); 1,40 (3H, s, CH_3); 1,37 (3H, s, CH_3); ^{13}C -NMR : 170,6 (2C of 2O**C**OCH_3); 156,7 (C_{12a}); 140,1 (C_{11a}); 138,6 (C_{10a}); 133,8 (C_9); 123,5 (C_5); 122,8 (C_7); 122,0 (C_8); 121,5 (C_{6a}); 117,3 (C_{10}); 115,1 (C_{5a}); 113,7 (C_{4a}); 102,8 (C_{12}); 78,6 (C_2); 68,7 (C_3); 65,0 (C_4); 24,7 and 24,1 (2 **C**H_3); 21,1 and 20,8 (2C of 2 OCO**C**H_3); $C_{21}H_{21}NO_6S$; Mass: (GC/MS) *m/z*: 454 ([MK]$^+$);

(±)-*cis*-12*H*-1,2-Diacetate-3,3-dimethyl-3*H*-pyrano[3,2-*a*]phenothiazine S-oxyd (22): IR: (KBr) $\sqrt{}_{max}$ (cm^{-1}): 3373 ; 1758 ; 1711 ; 1618; 1466; 1267; 1234; 1154; 1133; 1064; 1040; 750; ^{1}H -NMR: (DMSO-d_6), δ(ppm): 9,40 (1H, s, NH); 7,91 (1H, d, *J*=8 Hz, H_8) ; 7,84 (1H, d, *J*=9 Hz, H_6); 7,66 (1H, t, *J*=8 Hz, H_{10}); 6,78 (1H, d, *J*=8 Hz, H_{11}); 7,29 (1H, t, *J*=8 Hz, H_9); 6,78 (1H, s, H_5); 6,32 (1H, d, *J*=3 Hz, H_1); 5,57 (1H, d, *J*=3 Hz, H_2); 2,07 (3H, s, $OCOCH_3$); 2,05 (3H, s, $OCOCH_3$); 1,40 (3H, s, CH_3); 1,38 (3H, s, CH_3); ^{13}C -NMR : 170,8 and 170,3 (2C of 2 $OCOCH_3$); 157,5 (C_{4a}); 138,8 (C_{11a}); 138,5 (C_{12a}); 133,6 (C_{10}); 125,3 (C_6); 122,8 (C_8); 122,5 (C_9) ; 121,8 (C_{7a}); 118,9 (C_{11}); 115,0 (C_{6a}); 112,5 (C_5); 104,5 (C_{12b}); 77,4 (C_3); 68,9 (C_2); 64,6 (C_1); 24,0 et 23,8 (2C of 2 OCO**C**H_3); 21,4 and 21,0 (2 **C**H_3); $C_{21}H_{21}NO_6S$; Mass: (GC/MS) *m/z*: 454 ([MK]$^+$);

(±)-*cis*-12*H*-1,2-Diacetate-3,3-dimethyl-3*H*-pyrano[3,2-*a*]phenothiazine (23) IR: (KBr) $\sqrt{}_{max}$ (cm^{-1}): 3356, 1752, 1719, 1610, 1571, 1459, 1438, 1371, 1268, 1247, 1223, 1157, 1042, 746; ^{1}H -NMR: (DMSO-d_6), δ(ppm): 7.29 (1H, s, NH); 7.05 (1H, t, *J*=8 Hz, H_{10}); 7.00 (1H, d, *J*=8 Hz, H_8); 6.92 (1H, d, *J*=9 Hz, H_6); 6.85 (1H, t, *J*=8; 2 Hz, H_9); 6.68 (1H, d, *J*=8 Hz, H_{11}); 6.37 (1H, d, *J*=9 Hz, H_5); 6.20 (1H, d, *J*=3 Hz, H_1); 5.30 (1H, d, *J*=3 Hz, H_2); 2.09 (3H, s, $OCOCH_3$); 2.06 (3H, s, $OCOCH_3$); 1.32 (6H, s, 2CH_3); ^{13}C -NMR : 171.2 (2 $OCOCH_3$), 153.5 (C_{4a}), 142.0 (C_{12a}), 141.9 (C_{11a}), 128.5 (C_{10}), 127.9 (C_6), 126.7 (C_8), 123.4 (C_9), 118.7 (C_{7a}), 116.0 (C_{11}), 111.2 (C_5), 109.5 (C_{12b}), 105.4 (C_{6a}), 75.8 (C_3), 69.9 (C_2), 63.7 (C_1), 24.8, 23.1 (2 $OCOCH_3$), 21.3, 20.9 (2CH_3); $C_{21}H_{21}NO_5S$; Mass: (GC/MS) *m/z*: 399 ([M]$^+$);

3.2. Bioactivity:

The antimycobacterial activity was screened on Mycobacterium bovis BCG using the new Microdilution resazurin assay[17] (table 1).

The most promising compounds (8, 20, 23) were evaluated on the virulent strain Mycobacterium tuberculosis H37Rv by the same method[17] (table 2).

Table 1. Antimycobacterial of the pyranophenothiazine derivatives on M. bovis BCG

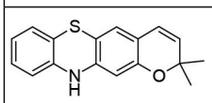
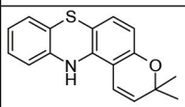
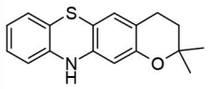
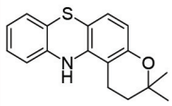
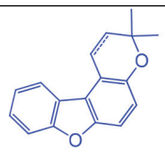
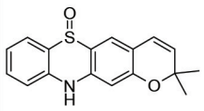
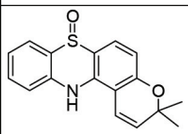
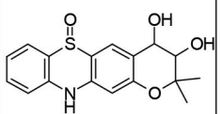
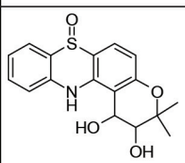
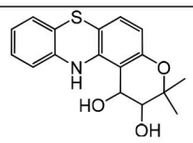
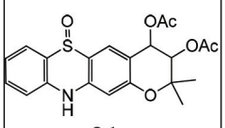
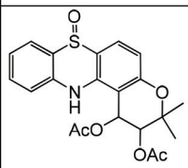
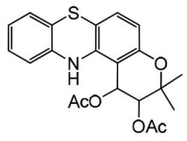
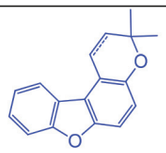
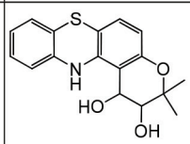
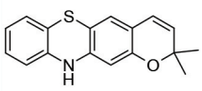
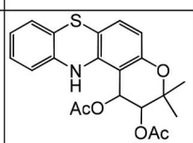
Compound	CMI_{95} (µg/ml)	Compound	CMI_{95} (µg/ml)	Compound	CMI_{95} (µg/ml)
 **8**	25	 **9**	>100	**INH**	**0,4**
 **14**	>100	 **15**	>100	 **7**	**10**
 **16**	100	 **18**	>100		
 **17**	>100	 **19**	>100	 **20**	25
 **21**	>100	 **22**	>100	 **23**	25

Table 2. Antimycobacterial of the pyranophenothiazine derivatives on M. tuberculosis

Compound	CMI_{95} (µg/ml)	Compound	CMI_{95} (µg/ml)
INH	**0,25**		
 **7**	**10**	 **20**	6,2
 **8**	6,2	 **23**	6,2

4. DISCUSSION

Based on previous synthetic experiments in the acronycine series[18], the synthesis of 8 and 9 was envisioned through Claisen rearrangement of an intermediate dimethylpropargyl ether. Thus, treatment of 10*H*-2-hydroxyphenothiazine (10) prepared from the commercially available 10*H*-2-methoxyphenothiazine (11) by a demethylation using boron tribromide (1*M* solution in methylene chloride) with 3-chloro-3-methylbut-1-yne (12)[19] in alkaline medium afforded the required 2-(1,1-dimethylpropargyloxy)-phenothiazine (13) in 65% yield (Scheme 1).

Thermal cyclization, performed by heating 13 under reflux for 1 hr in anhydrous dimethylformamide, afforded the two desired compounds 11*H*-2,2-dimethyl-2*H*-pyrano[2,3-*b*] phenothiazine (8) and 12*H*-3,3-dimethyl-3*H*-pyrano[3,2-*a*]phenothiazine (9). These products were separated by a silica gel column with cyclohexane - dichloromethane as mobile phase and obtained a total yield of 60% and 30%, respectively. The efficiency of this cyclization depended on the temperature, but the proportion of 2 products rested unchanged.

Hydrogenation of the compounds 8 or 9 conducted in ethanol, under H_2 using Pd-C (10%) as catalyst led to 11*H*-2,2-dimethyl-2*H*-3,4-dihydropyrano[2,3-*b*]phenothiazine (14) or 12*H*-3,3-dimethyl-3*H*-1,2-dihydropyrano [3,2-*a*]phenothiazine (15) in almost quantitative yield.

In order to modulate the lipophilicity in these two new series and to develop the structure-activity relationships, various modifications were conducted on the pyran ring and on the sulphur atom of the phenothiazine. Catalytic osmium tetroxide oxidation of 8 and 9 using *N*-methylmorpholine *N*-oxide to regenerate the oxidative agent[20], after a separated column, afforded the respective (+)-*cis*-diol sulfoxide (17) and (19) in 65 % and 80% yield respectively, accompanied by small amounts of the corresponding sulfoxides non hydroxylated on the pyran ring (16) and (18). In the angular series, a very small amount of (+)-*cis*-diol non-sulfoxide (20) were also obtained from the reaction mixture with the previously described reaction. Some other oxidative ways were carry out in order to prepare the (+)-*cis*-diol non-sulfoxide in series linear but not successfully, maybe because of its instability.

Further derivatisations were carried out. Treatment of the (+)-*cis*-diol compounds (17, 19, 20) with excess acetic anhydride gave the corresponding diacetates (21, 22, 23)

At this part, 12 new pyranophenothiazine derivatives had been prepared. Their structures had been confirmed by spectra of ^{1}H - NMR, ^{13}C- NMR, MS.

In fact, the molecular mass of each compound corresponded to its molecular structure.

All ^{1}H - NMR spectra contained the numbers of proton corresponding to their structures, with 2 peaks of 3H at about 1.3 ppm, corresponding to 2 methyl groups on pyran ring; 6 peaks 1H in the aromatic zone (from 8 to 5 ppm) attributed to the 6 proton of the phenothiazine ring; and 1 large single peak rather far from other peaks, about 10-9 ppm, corresponding to proton of NH on phenothiazine ring.

The spectra of 3 diol compounds (17, 19, 20) showing 2 peaks, which had disappeared with D_2O, confirmed the presence of 2 hydroxyl groups. The presence of diacetate groups on the spectra of 3 derivatives 21, 22, 23 were confirmed by 2 single peaks of 3H at about 2 ppm and the disappearance of 2 peaks of the 2 hydroxyl groups in comparison with the spectra of the corresponding diol.

All structures were confirmed by respective ^{13}C - NMR spectra.

The bioactivity of synthesized compounds was tested on *Mycobacterium bovis* BCG. From the results shown in Table 1, only the leading compound of linear series (8) and 2 derivatives 20 and 23 [(+)-*cis*-diol and (+)-*cis*-diacetate] of angular series demonstrated anti-mycobacterial activity with minimum inhibitory concentrations (MIC_{95}) on *Mycobacterium bovis BCG* at 25 µg/mL. These most active derivatives were also evaluated on *Mycobacterium tuberculosis* H37Rv (table 2). Their activities were more potent on this strain with MIC_{95} of 6.2 µg/mL and equivalent of those of the initial hits 3,3-dimethyl-3*H*-benzofuro[3,2-*f*] [1]-benzopyran 7 and its reduced analogue (MIC_{95} of 10 µg/ml) previously prepared by our collaborators[14].

These results showed that linearly fused compounds could be more potent than the angularly fused one. Compound 8 exhibited an interesting activity whereas its angular counterpart 9 was inactive. Any ways, the (+)-*cis*-diol and (+)-*cis*-diacetate derivatives of angular series presented an activity comparable with compound 8 of linear series promising an favorable activity of (+)-*cis*-diol and (+)-*cis*-diacetate derivatives in linear series.

In both series, all sulfoxide derivatives were devoid of activity. This could be explain by the drastic loss of lipophilicity (CLogP of sulfoxyde 16: 2.7 versus ClogP of 8: 5.1), which was in good agreement with previous observations for major antitubercular drugs, especially due to the lack of penetration of compounds on the mycobacterial cell wall.

5. CONCLUSION

In conclusion, 12 new pyranophenothiazine derivatives had been prepared. Their structures were confirmed by analysis of suitable spectral data (^{1}H-NMR, ^{13}C-NMR and MS). Their anti-bacterial activity was evaluated on *Mycobacterium bovis BCG*. Three most active compounds (8, 20, 23) were tested on the virulent *Mycobacterium tuberculosis* H37Rv. As a general rule, active derivatives were more potent on *Mycobacterium tuberculosis* than on *M. bovis* indicating a good selectivity. These structural requirements will be taken into account for the design of further analogues in pyranophenothiazine series.

6. ACKNOWLEDGEMENT

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REFERENCE

1. Who, *Global tuberculosis report 2016.* 2017.
2. Kumar D., Negi B., and Rawat D.S., *The anti-tuberculosis agents under development and the challenges ahead.* Future Med Chem, 2015. **7**(15): p. 1981-2003.
3. Gandhi N.R., Shah N.S., Andrews J.R., Vella V., Moll A.P., Scott M., et al., *HIV*

coinfection in multidrug- and extensively drug-resistant tuberculosis results in high early mortality. Am J Respir Crit Care Med, 2010. **181**(1): p. 80-6.

4. Gupta R.R., ed. *Phenothiazines and 1,4-benzothiazines, chemical and biomedical aspects*. Vol. 4. 1988: Amsterdam - Oxford - New York - Tokyo.
5. Amaral L., Kristiansen J.E., Viveiros M., and Atouguia J., *Activity of phenothiazines against antibiotic-resistant Mycobacterium tuberculosis: a review supporting further studies that may elucidate the potential use of thioridazine as anti-tuberculosis therapy.* J Antimicrob Chemother, 2001. 47(5): p. 505-11.
6. Martins M., Viveiros M., and Amaral L., *Enhanced Killing of Intracellular Pathogenic Bacteria by Phenothiazines and the Role of K+ Efflux Pumps of the Bacterium and the Killing Macrophage* Anti-Infective Agents in Medicinal Chemistry, 2008. **7**: p. 63-72.
7. Amaral L. and Molnar J., *Mechanisms by which thioridazine in combination with antibiotics cures extensively drug-resistant infections of pulmonary tuberculosis.* In Vivo, 2014. 28(2): p. 267-71.
8. Van Ingen J., *The broad-spectrum anti-mycobacterial activities of phenothiazines, InVitro: somewhere in all of this there may be patentable potentials.* Recent Pat Antiinfect Drug Discov. 6(2): p. 104-9.
9. Nicolaou K.C., Pfefferkorn J.A., Barluenga S., Mitchell H.J., Roecker A.J., and Cao G.-Q., *Natural product-like combinatorial libraries based on privileged structures. 1. General Principes and Solid-Phase Synthesis of Benzopyrans.* J. Am. Chem. Soc.,, 2000. 122: p. 9939-9953.
10. Bandara B.M.R., Hewage C.M., Karunaratne V., Wannigama G.P., and Adikaram N.K.B., *An antifungal chromene from Eupatorium riparium.* Phytochemistry, 1992. 31(6): p. 1983-1985.
11. Rao E.V., Sridhar P., Rao B.V.L.N., and Ellaiah P., *A prenylated dihydroflavonol from Mundulea suberosa.* Phytochemistry, 1999. **50**(8): p. 1417-1418.
12. Doan Thi Mai H., Gaslonde T., Michel S., Tillequin F., Koch M., Bongui J.B., et al., *Structure-activity relationships and mechanism of action of antitumor benzo [b]pyrano[3,2-h]acridin-7-one acronycine analogues.* J Med Chem, 2003. 46(14): p. 3072-82.
13. Michel S., Gaslonde T., and Tillequin F., *Benzo[b]acronycine derivatives: a novel class of antitumor agents.* Eur J Med Chem, 2004. 39(8): p. 649-55.
14. Prado S., Ledeit H., Michel S., Koch M., Darbord J.C., Cole S.T., et al., *Benzofuro [3,2-f][1]benzopyrans: a new class of anti-tubercular agents.* Bioorg Med Chem, 2006. 14(15): p. 5423-8.
15. Prado S., Janin Y.L., Saint-Joanis B., Brodin P., Michel S., Koch M., et al., *Synthesis and antimycobacterial evaluation of benzofurobenzopyran analogues.* Bioorg Med Chem, 2007. 15(5): p. 2177-86.
16. Bycroft B.W., Johnson A.P., and Landon W., *The reaction of skatole with 3-chloro-3-methylbut-1-yne: a novel ring expansion involving an allenic carbene.* J. Chem. Soc., 1969. D: p. 463a.
17. Palomino J.C., Martin A., Camacho M., Guerra H., Swings J., and Portaels F., *Resazurin microtiter assay plate: simple and inexpensive method for detection of drug resistance in Mycobacterium tuberculosis.* Antimicrob Agents Chemother, 2002. 46(8): p. 2720-2.
18. Hlubucek J., Ritchie E., and Taylor W.C., *A synthesis of acronycine.* Chem Ind, 1969. 50: p. 1809.
19. Bycroft B.W., Johnson A.P., and Landon W., *The reaction of skatole with 3-chloro-3-methylbut-1-yne: a novel ring expansion involving an allenic carbene.* J. Chem. Soc. D, 1969: p. 463a.
20. Vanrheenen V., Kelly R.C., and Cha D.Y., *An improved catalytic OsO4 oxidation of olefins to cis-1,2-glycols using tertiary amine oxides as the oxidant* Tetrahedron Letters, 1976. **17**(23): p. 1973-1976.