Arch Pharm Res Vol 27, No 7, 713-719, 2004

Archives of Pharmacal Research
http://apr.psk.or.kr

Synthesis and Antitubercular Activity of 6-Chloro (Unsubstituted)-2-Methoxy-9-Substituted Acridine Derivatives

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(Received February 23, 2004)

Several analogues of the general formulae 2-methoxy-9-substituted acridine and 6-chloro-2-methoxy-9-substituted acridine were synthesized and evaluated *in vitro* at 6.25 μg/mL against *M. tuberculosis* $H_{37}Rv$. Compounds **15** and **17** showed potential antitubercular activity with 100% inhibition to the virulent mycobacterium.

Key words: Acridine, Tuberculosis

INTRODUCTION

The World Health Organization (WHO) estimated that tuberculosis affects 1.7 billion people per year worldwide, killing *ca.* 3 million annually. It is estimated that 8 million new cases of tuberculosis (TB) emerge annually (Dye *et al.,* 1999; O'Brien and Nunn, 2001). The coincidence of tuberculosis with the AIDS epidemic is an additional problem (Ellner *et al.*, 1991). For cancer patients, the intensive use of immunosuppressants, steroids, radiation and surgical intervention were found as participating factors for TB infection. The death among those cancer patients is attributed mainly to TB rather than cancer (Yamada *et al.*, 1992).

The increasing resistance of *Mycobacterium tuberculosis* to currently available single and/or combined treatment and the spread of epidemic infections due to *Mycobacteria* are additional stimulating factors in search of new active compounds, particularly prototype leads (Iseman, 1993; Farmer and Kim, 1998).

Acridine derivatives are known for their antibacterial activity. In addition, there are numerous reports about their use as fluorescent tags to mycobacterial DNA and their use to reduce the development of resistance to antimycobacterial agents (Evans *et al.*, 1992, Alberghina and Palermo, 1975). However, there are only few reports about the use of acridines as antimycobacterial agents *per se*. Moreover, recent reports (Baca *et al.*, 2000; Thiim and Friedman, 2003; Zachariah *et al.*, 2003) showed an important positive impact of the -sulfonamide containing-cotrimoxazole combination in treating meningitis TB and TB associated with HIV. The suggested mechanism of action to the latter medication is different from that of acridines and includes depletion of the folate pool by inhibiting the enzyme 7,8-dihydropteroate synthase (DHPS). Also, many semicarbazone and thiosemicarbazone derivatives were reported to possess antitubercular action (Mir *et al.*, 1991).

In this article we present the synthesis and *in vitro* antimycobacterial activity of new acridine derivatives bearing sulfonamide, semicarbazide and thiosemicabazide moieties on position 9. The combination of moieties with more than one mechanism of action in a single chemical entity is expected to produce molecules to which the mycobacterium is less likely to develop resistance.

MATERIALS AND METHODS

Chemistry

Melting points were determined on the Electrothermal Melting Point apparatus and were uncorrected. Infrared spectra were recorded on the Shimadzu-470 infrared spectrophotometer. ^{1}H-NMR spectra were recorded in DMSO-d_6 on Varian XL-300 MHz or Joel 90 MHz spectrometers (chemical shifts are given in parts per million (PPM) downfield from TMS. Elemental analyses (C, H, N) were performed by the Microanalytical Unit, Faculty of

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Science, Cairo University; the values were found to be within ±0.4% of the theoretical ones, unless otherwise indicated. Mass spectra were made on the Hewlett Packard GC-MS, model 5890, series II. Compounds **4-7** (Ismail and Koreish, 2000; Kimura *et al*, 1989) and **23** (Omar and Hamouly, 1996) were prepared by reported procedures.

General procedure for the synthesis of compounds 8-21

The appropriate sulfonamide (0.003 mol) and 9-chloro-2-methoxy-acridine **6** (0.73 gm, 0.003 mol) or 6,9-dichloro-2-methoxyacridine **7** (0.83 gm, 0.003 mol) were stirred together in acetone for 1 h at room temperature in the presence of few drops of HCl. The mixture was poured onto ice-H_2O and neutralized with dilute ammonium hydroxide. The solid obtained was filtered, dried and crystallized from DMF.

4-[(2-Methoxyacridin-9-yl)amino]benzenesulfonamide (8)
Yield 91%; m.p. 245-246 °C; IR (KBr, Cm^{-1}): 3450, 3330; ^{1}H-NMR: 3.87 (s, 3H, OCH_3), 6.8-8.14 (m, 13H, aromatic + NH_2, exchangeable), 9.4 (brs, 1H, NH, exchangeable); MS *m/z*: 379 (100%); Anal. ($C_{20}H_{17}N_3O_3S$) C, H and N.

4-[(6-Chloro-2-methoxyacridin-9-yl)amino]benzenesulfonamide (9)
Yield 73%; m.p. 273-274 °C; IR (KBr, Cm^{-1}): 3480, 3350; ^{1}H-NMR: 3.87 (s, 3H, OCH_3), 6.85-8.02 (m, 10H, aromatic), 9.6 (brs, 1H, NH, exchangeable), 11.81 (brs, 2H, NH_2, exchangeable); MS *m/z*: 413 (100%); Anal. ($C_{20}H_{16}ClN_3O_3S$) C, H and N.

4-[(2-Methoxyacridin-9-yl)amino]-*N*-pyrimidin-2-ylbenzenesulfonamide (10)
Yield 94%; m.p. 268-269 °C; IR (KBr, Cm^{-1}): 3300; ^{1}H-NMR: 3.85 (s, 3H, OCH_3), 6.97-8.54 (m, 14H, aromatic), 9.6 (brs, 1H, NH, exchangeable), 11.86 (brs, 1H, NH, exchangeable); Anal. ($C_{24}H_{19}N_5O_3S$) C, H and N.

4-[(6-Chloro-2-methoxyacridin-9-yl)amino]-*N*-pyrimidin-2-ylbenzenesulfonamide (11)
Yield 82%; m.p. 265-267 °C; IR (KBr, Cm^{-1}): 3330; ^{1}H-NMR: 3.86 (s, 3H, OCH_3), 7.10-8.60 (m, 13H, aromatic), 9.6 (brs, 1H, NH, exchangeable), 11.30 (brs, 1H, NH, exchangeable); Anal. ($C_{24}H_{18}ClN_5O_3S$) H and N. calcd. C 58.6 found 59.8.

***N*-(4,6-Dimethyl-pyrimidin-2-yl)-4-(2-methoxyacridin-9-ylamino)benzenesulfonamide (12)**
Yield 91%; m.p. 262-264 °C; IR (KBr, Cm^{-1}): 3300, 3250; ^{1}H-NMR: 2.26 (s, 6H, 2×CH_3), 3.88 (s, 3H, OCH_3), 6.78-7.97 (m, 12H, aromatic), 9.5 (brs, 1H, NH, exchangeable), 11.30 (brs, 1H, NH, exchangeable); Anal. ($C_{26}H_{23}N_5O_3S$) C, H and N.

4-(6-Chloro-2-methoxyacridin-9-ylamino)-*N*-(4,6-dimethyl-pyrimidin-2-yl)benzenesulfonamide (13)
Yield 95%; m.p. 270-272 °C; IR (KBr, Cm^{-1}): 3330, 3250; ^{1}H-NMR: 2.25 (s, 6H, 2 x CH_3), 3.87 (s, 3H, OCH_3), 6.78-8.16 (m, 11H, aromatic), 9.6 (brs, 1H, NH, exchangeable), 11.40 (brs, 1H, NH, exchangeable); Anal. ($C_{26}H_{22}ClN_5O_3S$. 0.1 H_2O) C, H and N.

4-[(2-Methoxyacridin-9-yl)amino]-*N*-1,3-thiazol-2-ylbenzenesulfonamide (14)
Yield 92%; m.p. 205-207 °C; IR (KBr, Cm^{-1}): 3370, 3340; ^{1}H-NMR: 3.85 (s, 3H, OCH_3), 6.79-7.95 (m, 13H, aromatic), 9.6 (brs, 1H, NH, exchangeable), 11.40 (brs, 1H, NH, exchangeable); Anal. ($C_{23}H_{18}N_4O_3S_2$) C, H and N.

4-[(6-Chloro-2-methoxyacridin-9-yl)amino]-*N*-1,3-thiazol-2-ylbenzene sulfonamide (15)
Yield 85%; m.p. 245-247 °C; IR (KBr, cm^{-1}): 3370, 3340; ^{1}H-NMR: 3.87 (s, 3H, OCH_3), 6.80-8.23 (m, 12H, aromatic), 9.6 (brs, 1H, NH, exchangeable), 11.81 (brs, 1H, NH, exchangeable); Anal. ($C_{23}H_{17}ClN_4O_3S_2$) C, H and N.

4-(2-Methoxyacridin-9-ylamino)-*N*-(5-methyl-isoxazol-3-yl)benzenesulfonamide (16)
Yield 93%; m.p. 230-231 °C; IR (KBr, Cm^{-1}): 3370, 3340; ^{1}H-NMR: 2.31 (s, 3H, CH_3), 3.87 (s, 3H, OCH_3), 6.15 (s, 1H, 4-isoxazolyl), 6.81-8.09 (m, 11H, aromatic), 9.6 (brs, 1H, NH, exchangeable), 11.70 (s, 1H, NH); Anal. ($C_{24}H_{20}N_4O_4S$) C, H and N.

4-(6-Chloro-2-methoxyacridin-9-ylamino)-*N*-(5-methyl-isoxazol-3-yl)benzenesulfonamide (17)
Yield 94%; m.p. 250-251 °C; IR (KBr, Cm^{-1}): 3370, 3320; ^{1}H-NMR: 2.31 (s, 3H, CH_3), 3.88 (s, 3H, OCH_3), 6.15 (s, 1H, 4-isoxazolyl), 6.82-8.19 (m, 10H, aromatic), 9.72 (brs, 1H, NH, exchangeable), 11.30 (s, 1H, NH); Anal. ($C_{24}H_{19}ClN_4O_4S$) C, H and N.

***N*-[Amino(imino)methyl]-4-[(2-methoxyacridin-9-yl) amino] benzenesulfonamide (18)**
Yield 87%; m.p. 220-222 °C; IR (KBr, Cm^{-1}): 3450, 3320; ^{1}H-NMR: 3.20-3.65 (brm, 3H, NH_2 and NH), 3.85 (s, 3H, OCH_3), 6.69-8.10 (m, 11H, aromatic), 9.58 (brs, 1H, NH, exchangeable), 11.80 (s, 1H, NH); Anal. ($C_{21}H_{19}N_5O_3S$) C, H and N.

***N*-[Amino(imino)methyl]-4-[(6-chloro-2-methoxyacridin-9-yl)amino]benzenesulfonamide (19)**
Yield 92%; m.p. 290-291 °C; IR (KBr, Cm^{-1}): 3450, 3320;

^{1}H-NMR: 3.20-3.60 (brm, 3H, NH_2 and NH), 3.83 (s, 3H, OCH_3), 6.71-8.22 (m, 10H, aromatic), 9.9 (brs, 1H, NH, exchangeable), 11.90 (s, 1H, NH); Anal. ($C_{21}H_{18}ClN_5O_3S$) C, H and N.

N-({4-[(2-Methoxyacridin-9-yl)amino]phenyl}sulfonyl) acetamide (20)

Yield 78%; m.p. 215-217 °C; IR (KBr, Cm^{-1}): 3250, 1670; ^{1}H-NMR: 1.93 (s, 3H, CH_3), 3.86 (s, 3H, OCH_3), 6.80-8.24 (m, 11H, aromatic), 9.5 (brs, 1H, NH, exchangeable), 11.13 (s, 1H, NH, exchangeable); Anal. ($C_{22}H_{19}N_3O_4S$) C, H and N.

N-({4-[(6-Chloro-2-methoxyacridin-9-yl)amino]phenyl} sulfonyl)acetamide (21)

Yield 79%; m.p. 240-241 °C; IR (KBr, Cm^{-1}): 3250, 1670; ^{1}H-NMR: 1.93 (s, 3H, CH_3), 3.86 (s, 3H, OCH_3), 6.83-8.24 (m, 10H, aromatic), 9.65 (brs, 1H, NH, exchangeable), 11.2 (s, 1H, NH, exchangeable); Anal. ($C_{22}H_{18}ClN_3O_4S$) C, H and N.

General procedure for the synthesis of compounds 22-23

A mixture of 4-aminoacetophenone (0.4 gm, 0.003 mol) and 9-chloro-2-methoxy-acridine **6** (0.73 gm, 0.003 mol) or 6,9-dichloro-2-methoxyacridine **7** (0.83 gm, 0.003 mol) were refluxed in ethanol for 8 hours in the presence of few drops of HCl. The mixture was poured onto ice-H_2O and neutralized with dilute ammonium hydroxide. The solid obtained was filtered, dried and crystallized from alcohol.

1-{4-[(2-Methoxyacridin-9-yl)amino]phenyl}ethanone (22)

Yield 85%; m.p. 215-217 °C; IR (KBr, Cm^{-1}): 3420, 1690; ^{1}H-NMR: 2.16 (s, 3H, CH_3), 3.76 (s, 3H, OCH_3), 6.80-7.90 (m, 11H, aromatic), 9.65 (brs, 1H, NH, exchangeable); Anal. ($C_{22}H_{18}N_2O_2$) C, H and N.

1-{4-[(6-Chloro-2-methoxyacridin-9-yl)amino]phenyl} ethanone (23 (Omar and Hamouly, 1996))

General procedure for the synthesis of compounds 24-27

A mixture of **22** or **23** (0.002 mol) and semicarbazide (0.15 gm, 0.002 mol) or thiosemicarbazide (0.16 gm, 0.002 mol) was refluxed in absolute ethanol (25 mL) for 10 h and then cooled. The precipitate formed, was filtered, dried and crystallized from alcohol.

1-{4-[(2-Methoxyacridin-9-yl)amino]phenyl}ethan-1-one semicarbazone (24)

Yield 88%; m.p. 250-251 °C; IR (KBr, Cm^{-1}): 3520, 3450, 3400, 1670; ^{1}H-NMR: 2.11 (s, 3H, CH_3), 3.87 (s, 3H, OCH_3), 7.18-8.26 (m, 11H, aromatic), 9.50 (brs, 1H, NH, exchangeable), 9.80 (brs, 1H, NH, exchangeable), 11.70 (brs, 2H, NH_2, exchangeable); Anal. ($C_{23}H_{21}N_5O_2$) C, H and N.

1-{4-[(6-Chloro-2-methoxyacridin-9-yl)amino]phenyl} ethan-1-one semicarbazone (25)

Yield 85%; m.p. 210-211 °C; IR (KBr, Cm^{-1}): 3550, 3450, 3370, 1680; ^{1}H-NMR: 2.20 (s, 3H, CH_3), 3.77 (s, 3H, OCH_3), 7.18-8.22 (m, 10H, aromatic), 9.40 (brs, 1H, NH, exchangeable), 9.90 (brs, 1H, NH, exchangeable), 11.90 (brs, 2H, NH_2, exchangeable); Anal. ($C_{23}H_{20}ClN_5O_2$) C, H and N.

1-{4-[2-Methoxyacridin-9-yl)amino]phenyl}ethan-1-one thiosemicarbazone (26)

Yield 82%; m.p. 265-267 °C; IR (KBr, Cm^{-1}): 3400, 3250, 1650; ^{1}H-NMR: 2.29 (s, 3H, CH_3), 3.86 (s, 3H, OCH_3), 7.20-8.22 (m, 11H, aromatic), 9.80 (brs, 1H, NH, exchangeable), 10.10 (brs, 1H, NH, exchangeable), 11.70 (brs, 2H, NH_2, exchangeable); Anal. ($C_{23}H_{21}N_5OS$) C, H and N.

1-{4-[(6-Chloro-2-methoxyacridin-9-yl)amino]phenyl} ethan-1-one thiosemicarbazone (27)

Yield 80%; m.p. 220-221 °C; IR (KBr, Cm^{-1}): 3400, 3250, 1660; ^{1}H-NMR: 2.10 (s, 3H, CH_3), 3.87 (s, 3H, OCH_3), 7.21-8.27 (m, 10H, aromatic), 9.80 (brs, 1H, NH, exchangeable), 10.1 (brs, 1H, NH, exchangeable), 11.74 (brs, 2H, NH_2, exchangeable) ; Anal. ($C_{23}H_{20}ClN_5OS$) H and N. calcd. C: 61.39 found 61.90.

Biology

The final compounds **8-27** were screened *In vitro* at 6.25 μg/mL against *M. tuberculosis* H_{37}Rv (ATCC 27294; American Type Culture Collection, Rockville, MD) in BACTEC 12B medium, using the Microplate Alamar Blue Assay (MABA) (Collins and Franzblau, 1997; Suling *et al*, 2000). Rifampicin was used as the positive drug control (MIC = 0.25 μg mL^{-1}, 97% inhibition).

To minimize background fluorescence, antimicrobial susceptibility testing was performed in black, clear-bottomed, 96-well microplates (black view plates; Packard Instrument Company, Meriden, Conn.). Outer perimeter wells were filled with sterile water to prevent dehydration in experimental wells. Initial drug dilutions were prepared in either dimethyl sulfoxide or distilled deionized water, and subsequent two-fold dilutions were performed in 0.1 ml of 7H9GC (no Tween 80) in the microplates. BACTEC 12B-passaged inocula were initially diluted 1:2 in 7H9GC, and 0.1 mL was added to the wells. Subsequent determination of bacterial titers yielded 1×10^6 CFU/mL.

The frozen inoculum was initially diluted to 1:20 in the BACTEC 12B medium followed by a 1:50 dilution in

7H9GC. Adding 1/10 mL to the wells resulted in final bacterial titers of 2.0×10^5 CFU/mL for $H_{37}Rv$. The wells containing only the drug were used to detect autofluorescence of compounds. Additional control wells consisted of only the bacteria (B) and only the medium (M). were used

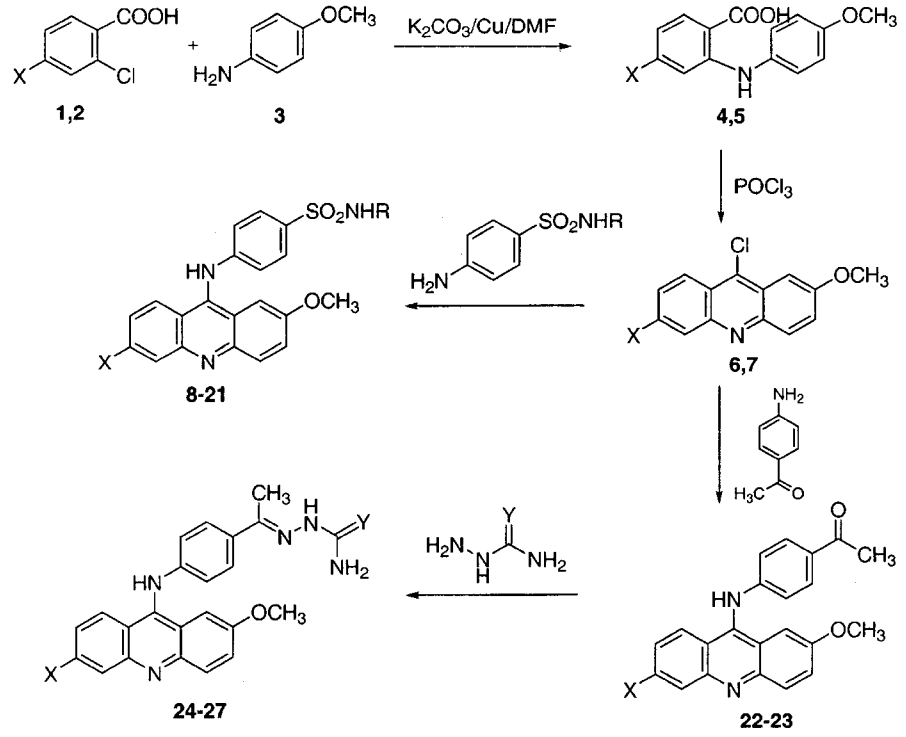
The plates were incubated at 37°C. Starting at day 4 of incubation, 20 µL of 10x alamar blue solution (Alamar Biosciences/Accumed, Westlake, Ohio) and 12.5 µL of 20% Tween 80 were added to one B well and one M well, and the plates were reincubated at 37°C. The wells were observed at the 12 h and 24 h marks for color change from blue to pink and for a reading of ≥50,000 fluorescence units (FU). Fluorescence was measured in a Cytofluor II microplate fluorometer (PerSeptive Biosystems, Framingham, Mass.) in the bottom-reading mode with excitation at 530 nm and emission at 590 nm. If the B wells became pink by 24 h, the reagent was added to the entire plate. If the well remained blue or ≤50,000 FU was measured, additional M and B wells were tested daily until a color change occurred, at which time reagents were added to all remaining wells. The plates were then incubated at 37°C, and results were recorded at 24 h. post-reagent addition. Visual MICs were defined as the lowest concentration of drug that prevented a color change. For fluorometric MICs, a background subtraction was performed on all wells with a mean triplicate M wells. Percent inhibition was defined as 1 – (test well FU/mean FU of triplicate B wells)×100. The lowest drug concentration effecting an inhibition of ≥90% was considered as the MIC.

RESULTS AND DISCUSSIONS

Chemistry

The synthetic pathways leading to the new acridine derivatives are illustrated in Scheme 1. The new target compounds **8-21** were prepared in good yields by stirring the appropriate sulfonamides, namely, sulfanilamide, sulfadiazine, sulfadimidine, sulfathiazole, sulfamethoxazole, sulfaguanidine and sulfacetamide, with 9-chloro-2-methoxyacridine **6** (Ismail and Koreish, 2000) or 6,9-dichloro-2-methoxyacridine **7** (Kimura *et al.*, 1989) in acetone and in the presence of a few drops of HCl. Also, condensation of **6** and **7** with 4-aminoacetophenone in the presence of HCl gave the acetylanilino derivatives **22, 23** (O'Brien and Nunn, 2001). Compounds **22** and **23** were condensed with semicarbazide and thiosemicarbazide giving the corresponding semicarbazone and thiosemicarbazone **24-27**, respectively.

As all the final compounds **8-27** can be considered as 9-anilinoacridine derivatives, they all showed a characteristic IR absorption band at around 3400 cm^{-1}, corresponding to



See Table I and experimental for X, Y and R

Scheme 1. Synthesis of acridine derivatives

the anilino NH function. Moreover, compounds **8** and **9** showed molecular ion peaks and base peaks both at *m/z* 379 and 413, respectively, indicating the stable nature of the synthesized derivatives.

Biology

All the final compounds **8-27** were screened *in vitro* at 6.25 μg/mL against *M. tuberculosis* $H_{37}Rv$. Fourteen out of the twenty tested compounds, showed antituberculotic activity; among them, two molecules, namely **15** and **17** demonstrated high levels of inhibition of *M. tuberculosis* (> 90%). The results are shown in Table I.

Nine out of 10 compounds with the 6-chloro substituent turned out to be more active than their non-chlorinated congeners. This rule was invalid only in the case of compound **20** relative to **21**. This highlights the impact of lipophilicity and the electronic properties of the 6-chlorine substituent upon the antitubercular activity.

Such a relation can be confirmed from the contrasting profiles of compound **15** (100% inhibition) in comparison to the total inactivity of its non-chlorinated derivative **14**.

Since the lipophilicity may be important for penetrating the bacterial cells, calculated log *P* values are given in Table I. Experimental log *P* was calculated by generating SMILES notations (ChemSketch software) for the respective compounds, followed by their use in the log *p* calculation (EPI software).

To check about the electronic properties of the chlorine substituent that may be involved in the antitubercular activity, the molecular electrostatic potential (MEP) maps (Hyperchem 6.0) for the geometrically optimized structures of compounds **14** and **15** were performed and compared to each other. The comparison showed a relation between the relative effectiveness of the compounds and the presence of an additional negative molecular electrostatic region (red) due to the chlorine substituent (Fig. 1).

Also, the lipophilicity may play a role in the relative antimycobacterial effectivness of the thiosemicarbazone

Table I. *In vitro* Antimycobacterium activity of compounds **8-27** at 6.25 μg mL^{-1} and their calculated log *P* values

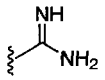
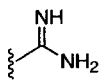
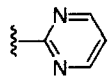
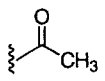
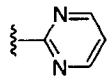
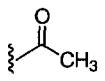
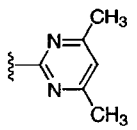
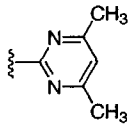
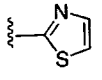
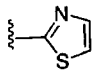
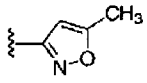
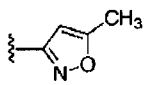
Cpd	X	Y	R	% inhibition	Log *P*	Cpd	X	Y	R	% inhibition	Log *P*
8	H	-	H	7	3.08	**18**	H	-		0	2.55
9	Cl	-	H	27	3.72	**19**	Cl	-		56	3.19
10	H	-		0	3.28	**20**	H	-		25	3.02
11	Cl	-		8	3.92	**21**	Cl	-		0	3.66
12	H	-		8	4.38	**22**	H	-	-	21	4.38
13	Cl	-		38	5.02	**23**	Cl	-	_	57	5.02
14	H	-		0	4.34	**24**	H	O	-	0	4.90
15	Cl	-		100	4.98	**25**	Cl	O	-	21	5.54
16	H	-		14	4.11	**26**	H	S	-	0	5.85
17	Cl	-		100	4.75	**27**	Cl	S	-	66	6.49

Fig. 1. The molecular electrostatic potential map of the geometrically optimised compounds **14** (left) and **15** (right), showing an additional negative (red) molecular electrostatic potential near the chlorine atom. The compounds were optimised by MM+ procedure and single point calculation was made by the AM1 semi-empirical method at a contour value of 0.015 a.u. ≈ (10 kcal/mol).

derivative **27** (66% inhibition) in comparison to its semicarbazone derivative **25** (21% inhibition).

Cyclization of the amidino function of the sulfaguanidine derivative **19** (56% inhibition) to its pyrimidenyl analogue **11** brought about a remarkable decrease in the antitubercular activity (8% inhibition); the non-chlorinated derivatives for both compounds **10** & **18** were devoid of biological activity. Also, the structurally related sulfadimidine derivative **13** (38% inhibition) was less active than the guanidine analogue **19**.

In conclusion, herein we report new acridine derivatives with potential activity against *M. tuberculosis per se*. The introduction of a 6- chlorine substitution seems crucial and advantageous for this activity. Some of the reported derivatives might represent good antitubercular leads. According to the TAACF regulations, compounds demonstrating at least 90% inhibition in the primary screen were serially diluted and re-tested at lower concentrations against *M. tuberculosis* $H_{37}Rv$ to determine the actual MIC, cytotoxicity (IC50) and the selectivity index (SI = IC50/MIC). Such results will be reported later.

ACKNOWLEDGEMENTS

The authors are grateful for the antimycobacterial data provided by the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) through a research and development contract with the US National Institute of Allergy and Infectious Diseases.

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