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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 5144–5147

Search of antitubercular activities in tetrahydroacridines: Synthesis and biological evaluation

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Received 17 April 2006; revised 4 July 2006; accepted 7 July 2006
Available online 25 July 2006

Abstract—A series of 9-substituted tetrahydroacridines were synthesized by nucleophilic substitution of chloro group with different nucleophiles in 9-chlorotetrahydroacridine (**2**). The latter could be obtained by $POCl_3$ mediated cyclization of the intermediate enamine, which in turn, was prepared by acid catalyzed condensation of anthranilic acid and cyclohexanone. Most of the compounds on antitubercular evaluation against *M. tuberculosis* H37 Rv and H37 Ra strains exhibited potent activities with MIC 6.125–0.78 μg/mL comparable to the standard drugs.

Tuberculosis (TB), caused by *M. tuberculosis*, is a major public health and socioeconomical problem in most of the developing countries.[1–3] It kills more than two million people every year worldwide in concurrence with HIV related infections.[4] About one-third of world's population is currently infected with this disease and the number is increasing every year. The coincidence of tuberculosis with the AIDS epidemic is an additional problem.[5] In cancer or other such disease where intensive use of immunosuppressants is required, the TB infection is aggravated and the death among such diseases is attributed mainly to TB rather than other diseases.[6] INH, rifampicin, ethambutol, pyrazinamide, and many other first and second line agents are known today to treat tuberculosis. The increasing incidence of the resistance to the currently available single and/or combined treatment and the spread of epidemic infections due to mycobacteria warrant the search of new active compounds particularly prototype leads[7,8] as novel therapies based on different mechanism of action. Acridine derivatives, atebrin and quinacrine (Fig. 1), have been widely used in malaria chemotherapy during world war-II in the absence of quinine[9a] and this skeleton is still being explored for better antimalarials.[9b] The antibacterial activities are also known to be associated with many acridine analogues and detail SAR in this class has been discussed recently.[10] It has been established that amino acridines having electronic conjugation between the ring nitrogen and the amino group are most active antibacterial agents.[11] It has also been documented that acridines reduce antimycobacterial resistance. However, there are only few reports about the use of acridines as antimycobacterial agent.[12,13]

Although phenoxazines, phenothiazines, and acridines are well-known pharmacophore for antitubercular activity, yet there is no report on the antimycobacterial profile of tetrahydroacridines. One of the proven mechanisms for biological action of acridine is their intercalation to nucleotide base pairs in the helix. Very recently, few acridines have been shown to inhibit DNA-coiling enzyme (topoisomerases) rather than DNA itself where the damage is caused by stabilization of the enzyme–DNA cleavage complex.[14] It was thought that if one of the condensed benzene ring is replaced with condensed tetrahydrobenzene the DNA intercalation may be affected. Although 9-amino-1,2,3,4-tetrahydroacridines (tacrine) are effective inhibitor of acetylcholine esterase useful in neurological disorders,[15] there is only one report where it has been mentioned that this class may be active against *Bacillus subtilis*[16]

Keywords: Tetrahydroacridines; Antitubercular agents; Tuberculosis.
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doi:10.1016/j.bmcl.2006.07.025

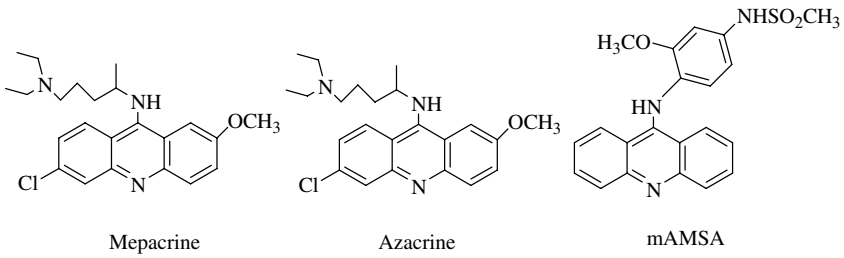


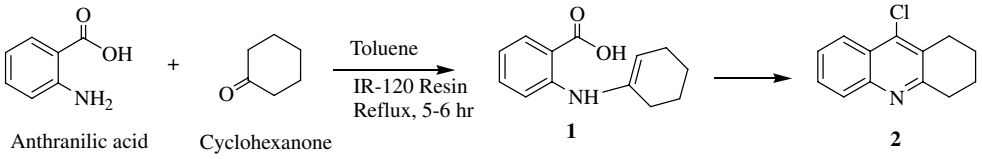
Figure 1. Few acridine derivatives used as drugs.

however, no activity is reported. Moreover, tetrahydroacridines have never been screened against *M. tuberculosis*. In addition, there are numerous reports about their use as fluorescent tags to mycobacterial DNA and to reduce the development of resistance to antimycobacterial agents.[17,18] Keeping the above facts in view and in continuation of our efforts to develop new antitubercular agents we were interested to synthesize 1,2,3,4-tetrahydroacridines having different substituents at C-9 and to assess the effect of these compounds on antitubercular profile (Schemes 1 and 2).

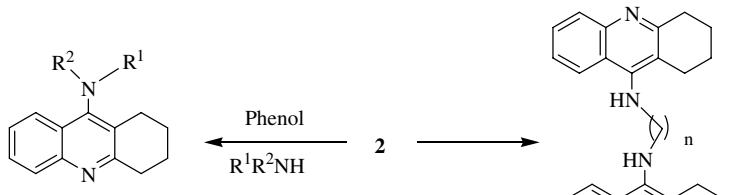
The intermediate 9-chloroacridine (**2**) was prepared by slight modification of the earlier reported method.[19] Thus, anthranilic acid and cyclohexanone were condensed in presence of dil HCl to give the intermediate enamine derivative (**1**), which on cyclization in presence of $POCl_3$ afforded 9-chloroacridine (**2**) in good yield.

The intermediate 9-chloroacridine (**2**) was reacted with different amines and phenols to give the respective desired products. Thus, reaction of 9-chloroacridine with different amines viz. *n*-hexyl amine, *n*-octyl amine, *n*-dodecyl amine, benzyl amine, (3-chlorophenyl)-ethyl amine, furylmethyl amine, anisidine, and morpholine in phenol[20] at 120–130 °C furnished respective 1,2,3,4-tetrahydro-9-aminoacridines (**3**–**10**) in very good yield. However, reaction of tetrahydroacridine **2** with 1,5-, 1,7-, and 1,10-diamines led to the formation of respective N^1,N^n-*bis*-(9-acridino)-diaminoalkanes (**11**–**15**) in good yields. The structures of compounds were established on the basis of spectroscopic data and analysis.[21]

The chloro group in chloroacridine intermediate **2** was also replaced with substituted phenoxy group by its reaction with different phenols viz. 4-chloro phenol, 2,4-dichloro phenol, 4-fluoro phenol, 3,4-dimethyl phenol, and 2-methoxy-4-allyl phenol resulting in respective 9-*O*-phenyl-tetrahydroacridines (**16**–**20**) (Scheme 3) in moderate to good yields. However, reaction of compound **2** with thiophenol resulted in respective 9-thiophenyl-tetrahydroacridine derivative (**21**).



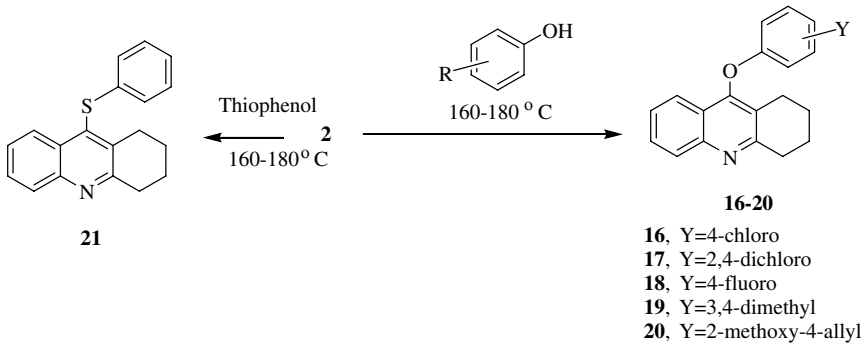
Scheme 1. Synthesis of 2-chloroacridine.



Compds	$-NR^1R^2$
3.	n-hexyl-NH-
4.	n-octyl-NH-
5.	n-dodecyl-NH-
6.	morpholinyl
7.	phenylmethylNH-
8.	3-chlorophenylethylNH-
9.	4-methoxy phenylNH-
10.	2-hydroxy ethylNH-

11, n=3
12, n=4
13, n=5
14, n=7
15, n=10

Scheme 2. Synthesis of 9-amino tetrahydroacridines.



Scheme 3. Synthesis of 9-(phenoxy and thiophenyl)-derivatives.

All the synthesized compounds were evaluated against *M. tuberculosis* H37 Ra and *M. tuberculosis* H37 Rv strains following earlier protocol.[22,23] Out of all the 20 compounds screened for anti-TB activities against *M. tuberculosis* H37 Ra and *M. tuberculosis* H37 Rv strains, nine compounds **3**–**6**, **10**, **11**, and **13**–**15** exhibited potent antitubercular activities with MIC varying from 12.5 to 0.78 μg/mL. Other compounds of the series were inactive as their MICs were above 12.5 μg/mL. A closure look into structure–activity relationship of the above compounds as evident from Table 1 reveals that tetrahydroacridines having 9-amino substituents are more active than those with thiophenyl or phenoxy substituents as their MIC values are >12.5 μg/mL. Further, among the 9-aminoalkyl acridines, compound **4** with eight-carbon chain length of *N*-alkyl substituent was most active against the avirulent strain *M. tuberculosis* H37 Ra with MIC of 1.56 μg/mL, while it has MIC of 3.125 μg/mL against the virulent strain *M. tuberculosis* H37 Rv. At the same time compound **5** with a chain of 12 carbon as the aminoalkyl substituent was the most potent compound of the series with MIC 0.78 μg/mL against H37 Rv strain, while it has MIC of 6.25 μg/mL against H37 Ra strain. These results indicate that compound **4** is more specific to avirulent strain H37 Ra, while compound **5** is more specific to the virulent strain H37 Rv. Instead of 9-aminoalkyl group, substitution with aromatic ring did not offer any significant inhibition to the bacterial growth. Among the *bis*-acridinyl diamino alkanes (**11**–**15**) compound **15** with 10-carbon chain spacer exhibited MIC of 3.125 μg/mL that too against avirulent strain, other compounds have MIC value either 12.5 or >12.5 μg/mL indicating that incorporation of one more acridine unit in such molecule is not beneficial. It is also evident from the activity data that replacement of aminoalkyl group at C-9 either with phenoxy (**16**–**20**) or thiophenyl (**21**) group results in complete loss of activity indicating that *N* atom at C-9 is essential for displaying antitubercular activity.

Table 1. In vitro antitubercular activities of synthesized tetrahydroacridines

Compound	MIC *M. tuberculosis H37 Ra*	MIC *M. tuberculosis H37 Rv*	log *P*
3	6.25	6.25	5.29
4	1.56	3.12	6.12
5	6.25	0.78	7.79
6	>25	12.5	3.60
7	25	>12.5	4.94
8	25	>12.5	5.78
9	>25	>12.5	5.05
10	>25	12.5	2.69
11	25	12.5	6.28
12	>25	ND	6.74
13	25	12.5	7.15
14	6.25	12.5	7.99
15	3.12	12.5	9.24
16	>25	>12.5	5.81
17	>25	>12.5	6.37
18	>25	>12.5	5.41
19	>25	>12.5	6.23
20	25	>12.5	6.18
21	>25	>12.5	5.82

MIC is defined as the lowest concentration inhibiting >90% or 90% of the inoculum relative to control against H37 Rv, MIC of the compounds used as control, INH 0.65, rifampicine 0.75, and ethambutol 3.25 μg/mL against H37 Rv. ND, not done.

In conclusion we have synthesized 9-substituted tetrahydroacridines as new series of antitubercular compounds with potent activity. The compound **5** having MIC as low as 0.78 μg/mL proved to be an excellent lead for further optimization and development.

Acknowledgments

We are thankful to Director, CDRI, Lucknow, for his encouragement to this work. S.S.V. and J.P. are thankful to UGC, New Delhi, and CSIR, New Delhi, for JRF.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.07.025.

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21. *9-Chloro-1,2,3,4-tetrahydroacridine* (**2**). A mixture of anthranilic acid and cyclohexanone was refluxed in toluene in presence of IR-120 resin with azeotropic removal of water for 5 h. The reaction mixture was filtered, the filtrate cooled, and the solid separated was filtered and crystallized from ethanol to give 2-(cyclohex-1-enylamino)benzoic acid (**1**) as colourless granules in 90% yield, mp 242–245 °C. To a cooled mixture of $POCl_3$ (50 mL) and toluene (50 mL), the above enamine **1**(10 g) was slowly added with vigorous stirring. The reaction mixture was brought to room temperature and stirring continued for 3 h. Now the reaction mixture was heated to 80–100 °C for additional 5 h with continuous stirring. The reaction mixture was cooled, poured into ice containing K_2CO_3 and the whole mixture was neutralized (pH 7) with aq ammonia. The reaction mixture was extracted with excess of toluene and the organic layer was evaporated under reduced pressure to give compound **2** as light yellow granules in 65% yield, mp 135–138 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.15, 7.97 (d, J = 8.4 Hz, each 1H, H-5, H-8), 7.67, 7.54 (dd, J = 8.4 Hz, 1.2 Hz each 1H, H-6, H-7), 3.11, 3.04 (m, 4H, H-1, H-4), 1.95 (m, 4H, H-2, H-3), FABMS: 217(M+H)$^+$; Anal. Calcd for $C_{13}H_{12}NCl$: C, 31.89; H,5.53; N, 6.45. Found: C, 31.74; H, 5.55; N, 6.41.
1,2,3,4-tetrahydro-9-(n-hexylamino)acridine (**3**). *Typical procedure*: A mixture of 9-chloroacridine **2** (1 g, 4.60 mmol), phenol (0.90 g, 9.20 mmol), and hexyl amine (0.70 mL, 0.69 mmol) was magnetically stirred initially at 80 °C for 3 h followed by 5 h at 130 °C untill the disappearance of compound **2**. The reaction mixture was cooled to room temperature and an excess of toluene was added. The reaction mixture was washed with 10% aq NaOH followed by saturated aq NaCl and finally with distilled water. The organic layer was separated, dried (Na_2SO_4), and evaporated to give crude material which was chromatographed over basic Al_2O_3 using 15% ethyl acetate/hexane as eluent to give **3** as colourless granules in 58% yield, mp 146–148 °C. IR(KBr): 3361 cm^{-1}; ^{1}H NMR(200 MHz, $CDCl_3$): δ 7.97, 7.91 (d, J = 7.5 Hz, 2H, H-5, H-8), 7.55, 7.34 (dd, 7.5 Hz, 1.2 Hz, 4H, H-6, H-7), 4.00 (br s, exchangeable 1H, NH), 3.48 (*t*, J = 4.8 Hz, 2H, CH_2NH), 3.06, 2.71 (m, 4H, H-1, H-4), 1.92 (m, 4H, H-2, H-3), 1.65 (m, J = 4.6 Hz, 2H, CH_2), 1.25–1.41(*m*, 6H, 3× CH_2), 0.89 (*t*, J = 4.2 Hz, 3H, CH_3), FABMS: 283(M+H)$^+$; Anal. Calcd for $C_{19}H_{26}N_2$: C, 80.85; H,9.22; N, 9.92. Found: C, 80.80; H, 9.28; N, 9.88. The physical data of other compounds may be seen in the supplementary data list.
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