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Synthesis and antituberculosis action of symmetric acridin-9-yl-bis-benzothiazol-2-yl-amines

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A new series of symmetric acridin-9-yl-bis-benzothiazol-2-yl-amines have been synthesized in good to excellent yields by intra-molecular cyclization of 1-acridin-9-yl-1-benzothiazol-2-yl-3-aryl thiourea and screened for their *in vitro* antitubercular activity against *Mycobacterium tuberculosis* by BACTEC radiometric method. The synthesis of new analogues bearing an acridine-linked chloro-substituted benzothiazole has demonstrated enhanced antituberculosis activity.

Keywords: Tuberculosis, Acridine, Benzothiazole

Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis* is responsible for the high mortality rate in most Asian countries. In 2019, As per WHO report, over 10 million new cases and nearly a million deaths cause of tuberculosis were reported[1]. It is estimated that more than 30% of the world's population is latently infected with tuberculosis and 1-2% of people have manifested active tuberculosis. Treatment for tuberculosis is commonly referred to as first-line treatment and typically lasts six months but can extend to nine months in some cases. In addition, failure to complete treatment increases the risk of developing drug-resistant tuberculosis[2,3]. An increasing number of tuberculosis patients in developing countries because their immune systems are weakened by immunosuppressive drugs or diseases such as AIDS. Drug-resistant strains of mycobacteria have emerged out so rapidly that almost 10% of cases are resistant to standard drugs and 3-5% are resistant to second-line drugs. The increased incidence of bacterial resistance to currently available antibacterial drugs necessitates the continued research in the field of drug design and development. The continued efforts for synthesis of new antitubercular drugs are highly encouraged.

Acridine is a planar molecule with high surface area and high ionization at neutral *p*H are key properties for to acts as an antibacterial agent[4]. In 1966 Adrien Albert showed that amino derivatives of acridine with electronic conjugation between nitrogen and amino group are the most active due to the high ionization tendency of the compounds[5]. In the acridine chromophore, the 9-position of ring carbon is important to act as an effective antibacterial agent[6] as well as the nitro/chloro analogs of acridine were used significantly as antimalarials. Some aminoacridine quaternary salts have also been studied as anticancer agents since its solubility in water is one of the important parameters for systematic use. The presence of amine group in acridine enhances possibility of combination of one or more biodynamic nucleus together.

The benzothiazole structure is an important scaffold for drug development due to various chemotherapeutic properties including larvicidal[7], anticancer agents[8], antidiabetics[9], in parasitic infections[10] and its analogues such as 2-mercaptobenzothiazole and 1,2,3-triazole-based bis-heterocyclics as an anti-inflammatory agents[11]. The benzothiazole derivatives with amino and chloro group have shown better anticancer activity[12]. Most extensively studied 2-aminobenzothiazole one of the privilege structures in medicinal chemistry owing to their utility as imaging agents for β-amyloid[13], as a photosensitizers[14,15], antifungal[16], antibacterial[17,18] and muscle relaxing agents[19]. Recently some new benzothiazole naphthyridone carboxylic acid derivatives are endowed with a high activity against multidrug-resistant tuberculosis demonstrate the importance of the benzothiazole nucleus as a biodynamic unit[20]. The idea of incorporating benzothiazole in medicinal compounds expanded the ability to combat fatal disease, reduce inflammation

and also improve the prognosis of patients diagnosed with HIV[21]. Similar to this work a series of 1*H*-1,2,3-triazole linked 7-chloroquinoline-pyrazolines were synthesized and evaluated for their antimycobacterial and cytotoxic activities[22].

Literature survey revealed that benzothiazole derivatives have been explored as potential anti-TB agents, the compound 2-(4-methoxyphenyl)-benzothiazole has shown potent anti-TB activity against both drug-sensitive and drug-resistant strains of *M. tuberculosis*. The reported 5-Chloro-2-phenyl-benzothiazoles have shown to exhibit anti-TB activity by inhibiting the growth of *M. tuberculosis*. It is believed to interfere with the bacterial cell wall synthesis[23].

Our interest was to link of benzothiazoles moiety to acridine nitrogen *via* formation and intramolecular cyclisation of 1-acridin-9-yl-aryl-thiourea[24]. Similarly second benzothiazole moiety was introduced by same route to get symmetric acridin-9-yl-bis-benzothiazol-2-yl-amines. Keeping in view of anti-tubercular efficiency of benzothiazoles, the current study was conducted to test the antituberculosis potential of acridin-9-yl-bis-benzothiazol-2-yl-amines using BACTEC method.

Results and Discussion

The representative compounds were prepared by intramolecular oxidative cyclization of 1-acridin-9-yl-1-benzothiazol-2-yl-3-aryl thiourea **5a-g** in acetic acid with bromine in acetic acid afforded acridin-9-yl-bis-aryl-benzothiazol-yl-amine **6a-g**. Initially compound 1-acridin-9-yl-3-aryl thiourea **4a-g** is prepared by mixture of 9-aminoacridine and N-aryl isothiocyanate **2a-g** in chloroform reflux for 2-3 hr. The significant advantages offered by this procedure are mild reaction condition, excellent yields of products. The product obtained after usual work-up showed single spot on TLC, recrystallized form absolute alcohol in cold condition.

The IR spectrum of the compound showed characteristic peak at 1594 cm^{-1}indicated -C=N group and ^{1}H NMR[26] spectrum of the compounds indicated signal at 7.4-7.9 ppm and 8-7.4 ppm for the aromatic ring of benzothiazole nucleus and acridine ring respectively. Mass spectra showed characteristic fragment ion peak at m/z 195 for acridinyl ring. Anti-mycobacterial screening results indicated that all compounds showed promising activity against *M. tuberculosis* when compared to standard drug rifampin (Table 1). Among the synthesized compounds **6d** was found to be the most active compound *in vitro* with MIC of 6.25 µM against mycobacteria.

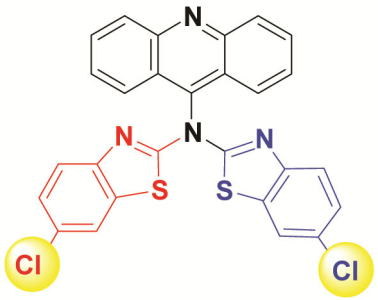
Experimental Section

Melting points were determined on a digital melting point apparatus (Veego VMP-D) and are uncorrected. The starting material (Sigma-Aldrich) and all chemicals used were of AR grade. Homogeneity of the compounds were checked on silica gel-G plates by TLC. The IR spectra were recorded on Perkin-Elmer spectrophotometer using KBr disc, ^{1}H NMR spectra were obtained on a Bruker Avance II 400 MHz spectrometer in $CDCl_3$ using tetramethyl silane as internal standard and Mass spectra were recorded on QTOF-Micromass spectrometer. Antituberculosis activity of title compounds were studied by BACTEC radiometric method[25].

Table 1 — Antituberculosis activity of compounds **6a-g**

Compd	BACTEC (Concentration in µM)				
	50	25	12.5	6.25	3.125
6a	S	S	S	R	R
6b	S	S	S	R	R
6c	S	S	S	R	R
6d	S	S	S	S	R
6e	S	S	S	R	R
6f	S	S	S	R	R
6g	S	S	S	R	R

S -Sensitive R -Resistance



Compound 6d with chloro group at 6-position showed inhibitory action against mycobateria at 6.25 µM

Synthesis of aryl isothiocyanates, 2a-g

In a round bottom flask, a mixture of concentrated ammonia (9 mL), pure carbon disulphide (5.4 g, 0.07 mol) and aniline (5.6 g, 0.06 mol) were taken, the flask stoppered and stirred well for about 1 h. The reaction mass was allowed to stand for 15-20 min. A heavy precipitate of salt of ammonium phenyl dithiocarbamate separated. To this salt, about 100-150 mL water was introduced with constant stirring and lead nitrate (20 g, 0.06 mol) solution was added. lead sulphide precipitated out. The mixture was steam distilled into receiver containing 2 mL (0.5M) sulphuric acid. From distillated solution, phenyl isothiocyanate as an oil was separated by separating funnel and dried over anhydrous calcium chloride. Similarly various aryl isothiocyanates **2a-g** were prepared by reported methods[27].

Synthesis of 1-acridinyl-3-aryl-thiourea, 3a-g

An equimolar mixture of acridin-9-yl-amine (4 mmol) and phenyl isothiocyanate **2a** (4 mmol) in chloroform was heated at reflux for 2-3 h. The reaction mixture was concentrated to approximately 2-3 mL and the resulting yellow precipitate of 1-acridin-9-yl-3-phenyl thiourea **3a** was filtered and washed with cold ethanol. The precipitate obtained was dried in desiccator and then checked till single spot showed on TLC plate of silica gel, and again washed with absolute alcohol to get pure product. Yield 80%, m.p. 182°C. R_f 0.72 (Benzene-Acetone, 9:1).

Synthesis of acridin-9-yl-benzothiazol-2-yl-amines, 4a-g

1-Acridinyl-3-phenyl-thiourea **3a** (2 mmol) was treated with excess of bromine (2 mmol) in acetic acid till the consumption of compound, resulting in brown acidic hydrobromide salt, which on basification with ammonia afforded free base. This was recrystallized with absolute ethanol. The precipitate obtained was dried in desiccator and formation of **4a** checked till single spot showed on TLC plate of silica gel. It was once again washed with absolute alcohol and identified as acridin-9-yl-benzothiazol-2-yl amine **4a**. Yield 88%, m.p. 218-220 (d) (Scheme 1).

Synthesis of acridin-9-yl-bis-benzothiazol-yl-amine, 6a-g

An equimolar mixture of 1-acridin-9-yl-benzothiazole-2-yl-amines **4a** (4 mmol) and phenyl isothiocyanates **2a** (4 mmol) in chloroform was heated at reflux for 2-3 h. The reaction mixture was concentrated to 2-3 mL. The resultant yellow precipitate of 1-acridin-9-yl-1-benzothiazol-2-yl-3-phenyl thiourea **5a** was washed with cold ethanol and dried in desiccator. Yield 88%, m.p. 162°C.

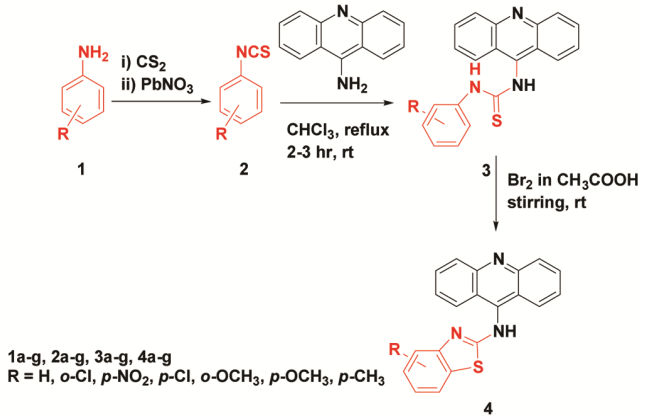
Further 1-acridin-9-yl-1-benzothiazol-2-yl-3-phenyl thiourea **5a** (2 mmol) on treatment with excess of bromine (2 mmol) in acetic acid till the consumption of compound, resulted in yellowish solid acidic to litmus. This, upon on basification with aqueous ammonia afforded free base and after washing with cold ethanol, identified as acridin-9-yl-bis-benzothiazol-yl-amine **6a**. Yield 83%, m.p. 212-213°C (d). R_f 0.60 (Benzene-Acetone, 9:1) (Scheme 2).

Spectral data of acridin-9-yl-bis-benzothiazol-2-yl amines, 6a-e

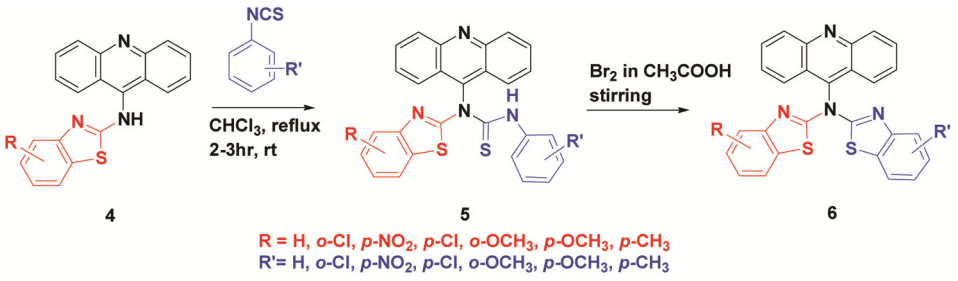
Acridin-9-yl-bis-benzothiazol-2-yl amines 6a: IR (KBr): 1549 (C=N), 1487 (C=C), 1165 cm^{-1} (C-N); ^{1}H NMR ($CDCl_3$): δ 7.6-7.9 (8H, m, acridine ring), 7.3-7.0 (10H, m, aromatic ring proton); ^{13}C NMR (DMSO-d_6): δ 140 (3C, s, acridine ring), 134 (3C, s, acridine ring), 123 (3C, s, acridine ring), 156 (2C, s, thiazole ring); MS (ESI): *m/z* 460 (M^+), 326, 195, 135.

Acridin-9-yl-bis-(4-chloro)-benzothiazol-2-yl amine 6b: IR (KBr): 3103 (C-H), 1549 (C=N), 1440 (C=C), 1167 cm^{-1} (C-N); ^{1}H NMR ($CDCl_3$): δ 7.8-8.1 (6H, m, acridine ring), 7.1-7.4 (6H, m, aromatic ring proton); ^{13}C NMR (DMSO-d_6): δ 140 (1C, s, acridine ring), 137 (6C, s, aromatic ring), 124 (3C, s, acridine ring) 154 (2C, s, thiazole ring); MS (ESI): *m/z* 528 (M^+), 326, 195.

Acridin-9-yl-bis-(6-nitro)-benzothiazol-2-yl amine 6c: IR (KBr): 3129 (C-H), 1592 (C=N), 1440 (C=C), 1165 cm^{-1} (C-N); ^{1}H NMR ($CDCl_3$): δ 7.7-7.9



Scheme 1



Scheme 2

(6H, m, acridine ring), 7.4-6.9 (10H, m, aromatic ring proton), 2.1(6H, s, methyl proton); ^{13}C NMR (DMSO-d_6): δ 140 (1C, s, acridine ring), 135 (3C, s, aromatic ring), 127 (3C, s, aromatic ring), 157 (2C, s, thiazole ring); MS (ESI): *m/z* 550 (M^+), 326, 195.

Acridin-9-yl-bis-(6-chloro)-benzothiazol-2-yl amine 6d: IR (KBr): 3103 (C-H), 1594 (C=N), 1487 (C=C), 1168 cm^{-1} (C-N); ^{1}H NMR ($CDCl_3$): δ 7.9-8.0 (8H, m, acridine ring), 7.2-7.6 (10H, m, aromatic ring proton), 2.3 (6H, s, methyl proton); ^{13}C NMR (DMSO-d_6): δ 140 (1C, s, acridine ring), 134 (3C, s, aromatic ring), 124 (3C, s, aromatic ring), 157 (2C, s, thiazole ring); MS (ESI): *m/z* 528 (M^+), 326, 195.

Acridin-9-yl-bis-(4-methyl)-benzothiazol-2-yl amine 6e: IR (KBr): 1595 (C=N), 1487 (C=C), 1165 cm^{-1} (C-N); ^{1}H NMR ($CDCl_3$): δ 7.9-8.1 (8H, m, acridine ring), 7.4-7.7 (10H, m, aromatic ring proton); ^{13}C NMR (DMSO-d_6): δ 140 (1C, s, acridine ring), 131 (3C, s, aromatic ring), 123 (3C, s, aromatic ring), 157 (2C, s, thiazole ring); MS (ESI): *m/z* 488 (M^+), 326, 195.

Acridin-9-yl-bis-(6-methoxy)-benzothiazol-2-yl amine 6f: IR (KBr): 3106 (C-H), 1592 (-NO_2), 1487 (C=C), 1168 cm^{-1} (C-N); ^{1}H NMR ($CDCl_3$): δ 7.9-8.1 (8H, m, acridine ring), 7.2-7.4 (10H, m, aromatic ring proton), 2.3 (s, 6H, methyl proton); ^{13}C NMR (DMSO-d_6): δ 140 (1C, s, acridine ring), 136 (3C, s, aromatic ring), 124 (3C, s, aromatic ring), 158 (2C, s, thiazole ring); MS (ESI): *m/z* 520 (M^+), 326, 195.

Antituberculosis activity of compounds 6a-g

All the compounds were evaluated for their *in vitro* anti-tubercular activity by BACTEC radiometric method for direct determination of the minimum inhibitory concentration (MIC) against *M. tuberculosis*. For the BACTEC assay test compounds were dissolved in 10% (v/v) DMSO at a concentration of 10 mM, the test vial sjnhjn of 7H12 medium containing ^{14}C labelled palmitic acid were inoculated with mycobacteria and incubated at 37°C temperature. The amount of $^{14}CO_2$ reflects the rate and amount of growth and is expressed in term of the "Growth Index" (GI). On addition of compound to the test vial, suppression of growth of the test organism *M. tuberculosis* could be detected by routine observation of Growth Index output as compared to the control and standard drug rifampin (2 μg/mL). We present herein the preliminary results concerning the synthesis and the *in vitro* antitubercular activity of representative compounds of this family.

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