International Journal of Drug Development & Research
| July-Sept 2010 | Vol. 2 | Issue 3 | ISSN 0975-9344 |
Available online http://www.ijddr.in
Covered in Official Product of Elsevier, The Netherlands

SYNTHESIS AND BIOLOGICAL EVALUATION OF 3-AMINO-2-CYANO-5-(SUBSTITUTED AMINO)-4-[(UN) SUBSTITUTEDPHENYL] THIOPHENES AS ANTI-TUBERCULAR AGENTS

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ABSTRACT

Tuberculosis, due to its relentless nature, is now a major public health threat. The concomitant resurgence of TB with the MDR- or XDR-TB and HIV/AIDS pandemic has exposed the frailties of the current drug armatorium. Based on good structural similarity between BM-212, a novel antimycobacterial agent undergoing clinical trials, and diaryl thiophenes, we have designed novel diaryl thiophenes. Alkyl or aryl isothiocyanates were reacted with substituted phenylacetonitrile in the presence of NaH to afforded 3-mercapto-3-(substitutedamino)-2-[(un)substitutedphenyl]acrylonitrile (5a-h). The designed molecules, 3-amino-2-cyano-5-(substitutedamino)-4-[(un)substitutedphenyl]thiophenes (6a-h) were synthesized by cyclocondention of 3-mercapto-3-(substitutedamino)-2-[(un)substitutedphenyl]acrylonitrile (5a-h) with chloroacetonitrile in ethanol. All the compounds were screened for their antimycobacterial activity on mycobacterium tuberculosis using H37Rv strain by 1% proportion method. Some of the synthesized compounds exhibited potent antimycobacterial activity with MIC values in the range of 12.5-100 µg/mL.

Key words: *anti tubercular, thiophene, isothiocyanates*

Introduction

Tuberculosis (TB), caused by Mycobacterium tuberculosis, is a disease rich in paradoxes. Currently, one third of the world population is latently infected with TB bacteria.[1] Despite the availability of the BCG vaccine and chemotherapy, TB still remains a leading infectious disease globally, especially in Third World countries. According to estimates of the World Health Organization (WHO), TB is now the leading infectious cause of death worldwide and there were an estimated 9.2 million new cases of TB every year [1] (afflicting mostly the young and productive adults). Because of relentless spread of TB throughout the world, WHO took the unprecedented step of declaring TB a global emergency in 1993 that has to be given prime importance.[2] The problem has worsened primarily due to the growing human immunodeficiency virus (HIV) epidemic and the emergence of drug resistance.[3] There were an estimated 1.5 million deaths from TB in HIV-negative people and 0.2 million among people infected with HIV.[1]

The approach to chemotherapy of TB is very different from that for other bacterial infections. The organism has a long generation time and a capacity for dormancy, when its low metabolic activity makes it a difficult therapeutic target.[4–6] In

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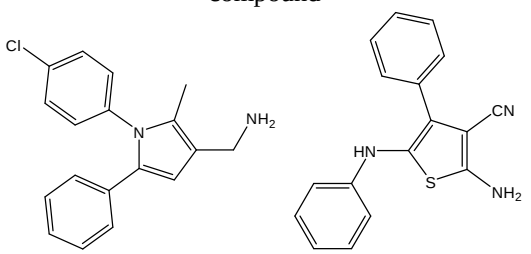
addition, M. tuberculosis may be located in pulmonary cavities, empyema pus, or solid caseous material, where penetration of antibiotics is difficult or the pH is sufficiently low to inhibit the activity of most antibiotics.[7,8] Although TB can be cured by an optimized regimen comprising of various first line and second line drugs,[9,10] the emergence of MDR-TB and extremely drug resistant TB (XDR-TB, first reported in November 2005[11] has created new challenges to control and defeat the disease. The concomitant resurgence of TB with the MDR- or XDR-TB and HIV/AIDS pandemic has exposed the frailties of the current drug armatorium. All these suggest that there is an urgent need of new potent therapeutic agents which can be effective against resistant strains of mycobacteria.

BM-212, an pyrole derivative[12] has generated considerable excitement with its antitubercular potency and presently is undergoing clinical trials. It was thought of interest to replace the pyrole ring of BM-212 with thiophene ring (Figure 1).This prompted us to synthesize a series of 3-amino-2-cyano-5-(substitutedamino)-4-[(un)substitutedphenyl]thiophenes and check its antimycobacterial potential.

Figure 1: comparison of BM-212 and targeted compound



BM-212 Targeted compound

Material and method:

Melting points of all compounds were determined in open capillaries and are uncorrected. TLC was performed on microscopic slides (2×7.5cms) coated with Silica-Gel-G and spots were visualized by exposure to iodine vapor. UV spectra were recorded in methanol double beam UV-VIS Pharmaspect 1700 Shimadzu spectrophotometer. IR spectra of all compounds were recorded in KBr (Merck) on FT-IR 8400S Shimadzu spectrophotometer. Mass spectra were recorded on SHIMADZU LCMS 2010 EV Mass Spectrometer. ^{1}H NMR spectra were obtained on BRUKER Advance-II 400 MHz instrument in $CDCl_3$ as solvent and chemical shift were measured as parts per million downfield from tetramethylsilane (TMS) as internal standard.

General procedure for synthesis of isothiocyanates (3a-f);

A 500 mL round bottom flask was charged with appropriate amines (16 mmol), triethylamine (12.2 mmol) and 30 mL benzene in stirring. Carbon disulphide (11.8 mmol) was added drop wise by syringe maintaining temperature to 0-5 °C. Light yellow colored dithiocarbamate salt was formed within 30 min. Reaction mass was kept in deep freeze for 72 h. Salt thus obtained was filtered under vacuum and washed with diethylether (30 mL x 3). The salt was dissolved in 40 mL chloroform followed by addition of triethylamine (12.2 mmol). Ethyl chloroformate (11.8 mmol) was added drop wise at temperature not exceeding 0 °C. The reaction was stirred at room temperature for 1 h followed by addition of 50 mL of 5 N HCl. Chloroform layer was separated and further dried with brine solution and eventually with anhydrous sodium sulphate. The organic layer was concentrated by downward distillation and residue was further vacuum distilled to obtain isothiocyanate **(3a-f)** as transparent liquid.

General procedure for synthesis of 3-mercapto-3-(substitutedamino)-2-[*(un)*substitutedphenyl]acrylonitrile (5a-h);

To a suspension of sodium hydride (2 mmol) in 20 mL benzene with cooling and stirring, (substituted)phenylacetonitrile (1 mmol) was added

at once. Alkyl or aryl isothiocyanates (1 mmol) in 8 mL DMF were added drop wise and stirred for 1 h. Reaction mixture was poured into water and benzene layer was separated. The aqueous layer was acidified with dilute hydrochloric acid under cooling condition to afforded yellow crystalline solid 5a-h.

General procedure for synthesis of 3-amino-2-cyano-5-(substitutedamino)-4-[(un) substitutedphenyl] thiophenes (6a-h);

To a solution of 2a-h (0.3 mmol) in 15 mL absolute alcohol, triethylamine (0.3 mmol) and chloroacetonitrile (0.3 mmol) was added with stirring. After 30 min, color of the solution was changed to dark green. The reaction mixture was poured in ice-cold water. Solid separated was filtered and dried to give 6a-h.

3-amino-2-cyano-4-phenyl-5-(propylamino)thiophene (6a): yellow crystals; mp 108-110°C; IR (KBr) 3314.65, 3434.65 (NH_2), 3292.26 (NH), 2975.31 (CH), 2173.67 (CN) cm^{-1}; ^{1}HNMR (300 MHz,$CDCl_3$) 1.0-1.1 (t, 3H, CH_3), 1.8-1.9 (m, 2H, CH_2), 3.7 (s, 2H, NH_2, D_2O exchangeable), 4.21-4.25 (q, 2H, NCH_2), 7.2-7.4 (m, 5H, Ar); MS, *m/z* (%): 257.1 (M+1).

3-amino-2-cyano-5-isopropylamino-4-phenylthiophene (6b): yellow crystals; mp 114-118°C; IR (KBr) 3321.21, 3452.34 (NH_2), 3301.91 (NH), 2966.31 (CH), 2189.27 (CN), cm^{-1}; ^{1}HNMR (300 MHz,$CDCl_3$) 1.6-1.7 (d, 6H, CH_3), 3.6 (s, 2H, NH_2), 5.1-5.2 (m, 1H, CH), 7.2-7.4 (m, 5H, Ar); MS, *m/z* (%): 257.1 (M+1).

3-amino-4-(4-chlorophenyl)-2-cyano-5-(phenylamino)thiophene (6c): yellow crystals; mp 108-112°C; IR (KBr) 3321.78, 3442.45 (NH_2), 3280.69 (NH), 2189.06 (CN), cm^{-1}; ^{1}HNMR (300 MHz,$CDCl_3$) 4.2 (s, 2H,NH_2), 7.1-7.6 (m, 9H, Ar), 8.1 (s, 1H, NH); MS, *m/z* (%):325.7 (M), 327.5 (M+2).

3-amino-2-cyano-4-phenyl-5-(p-toluidino)thiophene (6d): yellow crystals; mp 120-124°C; IR (KBr) 3345.54, 3449.87 (NH_2), 3278.25 (NH), 2925.81 (CH), 2187.13 (CN) cm^{-1}; ^{1}HNMR (300 MHz,$CDCl_3$) 2.6 (s, 3H, CH_3), 4.1 (s, 2H, NH_2), 7.2-7.6 (m, 9H, Ar), 8.1 (s, 1H, NH); MS, *m/z* (%): 305.8 (M+1).

3-amino-4-(4-chlorophenyl)-2-cyano-5-(4-toluidino)thiophene (6e): yellow crystals; mp 116-118°C; IR (KBr) 3325.65, 3467.78 (NH_2), 3280.69 (NH), 2954.87 (CH), 2189.06 (CN) cm^{-1}; ^{1}HNMR (300 MHz,$CDCl_3$) 2.5 (s, 3H, CH_3), 4.1 (s, 2H, NH_2), 7.1-7.6 (m, 8H, Ar), 8.1 (s, 1H, NH); MS, *m/z* (%): 339.8 (M), 341.8 (M+2).

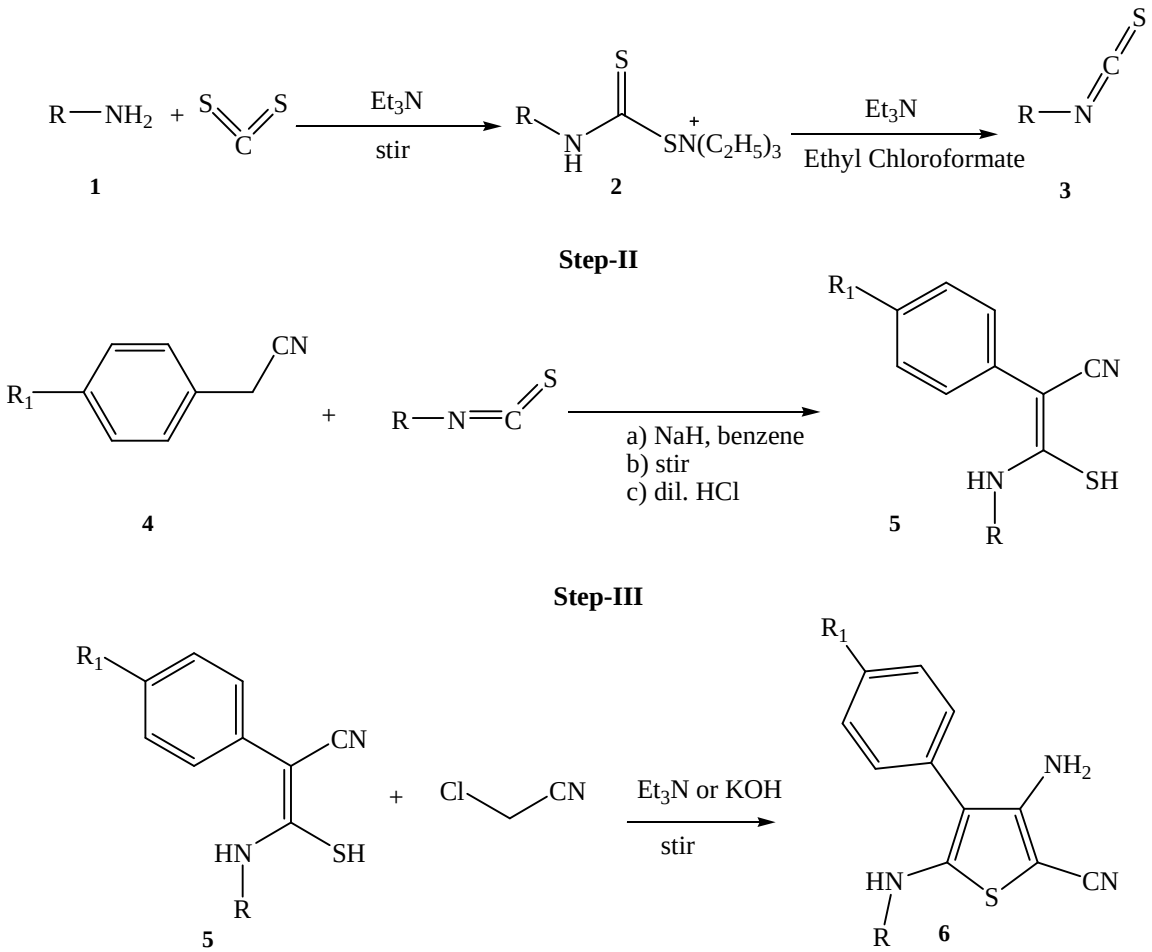
3-amino-2-cyano-5-(4-methoxyphenylamino)-4-phenylthiophene (6f): yellow crystals; mp 122-125°C; IR (KBr) 3321.34, 3451.55 (NH_2), 3290.69 (NH), 2968.24 (CH), 2181.06 (CN), cm^{-1}; ^{1}HNMR (300 MHz,$CDCl_3$) 3.9 (s, 3H, OCH_3), 4.0 (s, 2H, NH_2), 7.0-7.4 (m, 9H, Ar), 8.1 (s, 1H, NH); MS, *m/z* (%):321.8 (M+1).

3-amino-4-(4-chlorophenyl)-2-cyano-5-(4-methoxyphenylamino)thiophene (6g): yellow crystals; mp 110-114°C; IR (KBr) 3315.85, 3434.65 (NH_2), 3317.34 (NH), 2968.24 (C-H), 2189.06 (CN) cm^{-1}; ^{1}HNMR (300 MHz,$CDCl_3$) 3.9 (s, 3H, OCH_3), 4.0 (s, 2H, NH_2), 7.1-7.4 (m, 8H, Ar), 8.1 (s, 1H, NH).

3-amino-4-(4-chlorophenyl)-5-[(4-chlorophenyl)amino]-2-cyanothiophene (6h): yellow crystals; mp 100-102°C; IR (KBr) 3316.65, 3434.54 (NH_2), 3319.26 (NH), 2190.98 (CN) cm^{-1}; ^{1}HNMR (300 MHz,$CDCl_3$) 4.2 (s, 2H, NH_2), 7.2-7.6 (m, 8H,

Ar), 8.1 (s, 1H, NH); MS, *m/z* (%):359.7(M), 361.6 (M+2), 363.7 (M+4).

Figure 2 Reaction scheme for synthesis of design compounds (6a-h)



R = Ph, 4-Cl-Ph, 4-Me-Ph, 4-OMe-Ph, Isopropyl, n-propyl, R_1 = H, Cl

Anti-tubercular activity:

The test compounds were subjected to screening by Lowenstein Jensen Method[13] using *H37Rv* strain of *Mycobacterium tuberculosis***.**

Composition of modified L-J media

Potassium dihydrogen phosphate	1.2 g
Magnesium sulphate	0.12 g
Magnesium citrate	0.3 g
L-asparagine	1.8 g
Glycerol/Sodium pyruvate	6.0 mL/3.6 g
Distilled water	300 mL
Malachite green (2%)	16 mL
Egg homogenate	500 mL
Benzyl penicillin (1,000,000 IU/ml)	1 mL

Result and discussion:

Nucleophilic addition[14] of aryl or alkylamine **1** on carbon disulphide in presence of triethylamine gave the intermediate dithiocarbamate salt **2** which is decomposed by ethyl chloroformate to yield corresponding aryl or alkyl isothiocyanates **3**. (Substituted)phenylacetonitriles **4** were charged with sodium hydride in benzene to generate the carbanion.[15] This carbanion immediately attack on aryl or alkyl isothiocyanates **3** to afford 3-mercapto-3-[substitutedamino]-2-[(un)substitutedphenyl]acrylonitriles **5** in good yield. The targeted compounds **6** were synthesized by stirring 3-mercapto-3-(substitutedamino)-2-[(un)substitutedphenyl]acrylonitrile **5** with

chloroacetonitrile in alcohol in presence of triethylamine or potassium hydroxide at room temperature.

The test compounds were subjected to screening by Lowenstein Jensen Method using *H37Rv* strain of *mycobacterium tuberculosis.* The compounds were screened for anti tubercular activity at 12.5 µg/mL, 50 µg/mL and 100 µg/mL against *H37Rv* strain of *Mycobacterium tuberculosis* and data are recorded in table 1. All compounds were found to be active at 100 µg/mL concentrations except compound 6a. Compound 6f was found to be active at all concentration so its MIC will be < 12.5 µg/mL.

Table 1: Antitubercular activity of the targeted compounds

Comp No.	R_1	R	Concentration of compound		
			12.5 µg/mL	50 µg/mL	100 µg/mL
6a	n-propyl	H	Growth detected	Growth detected	No growth
6b	Isopropyl	H	Growth detected	Growth detected	No growth
6c	Phenyl	Cl	Growth detected	Growth detected	No growth
6d	4-methylPhenyl	H	Growth detected	Growth detected	No growth
6e	4-methylphenyl	Cl	Growth detected	Growth detected	Growth detected
6f	4-methoxyphenyl	H	No growth	No growth	No growth
6g	4-methoxyphenyl	Cl	Growth detected	Growth detected	No growth
6h	4-chlorophenyl	Cl	Growth detected	Growth detected	No growth

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Article History:----------

Date of Submission: 21-02-10

Date of Acceptance: 03-05-10

Conflict of Interest: None

Source of Support: Nil