Synthesis, antituberculosis activity and QSAR study of some novel 2-(nitroaryl)-5-(nitrobenzylsulfinyl and sulfonyl)-1,3,4-thiadiazole derivatives

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ABSTRACT

Background and the purpose of the study: In continuation of our research program for new antitubercular agents, some hybrid compounds containing a 5-nitrohetrocyle and 1,3,4-thiadiazole ring havae been synthesized and their antituberculosis activity have been evaluated. QSAR studies were subsequently used to find the structural requirements for activity of this series of compounds.

Methods: 2-(nitroaryl)-5-(nitrobenzylsulfinyl and sulfonyl)-1,3,4-thiadiazole derivatives, have been synthesized and evaluated against *Mycobacterium tuberculosis* H_{37}Rv (ATCC27294) in BACTEC 12B medium using a broth micro dilution assay. The minimum inhibitory concentration (MIC) was determined for compounds that demonstrated $\geq 90\%$ growth inhibition in the primary screening. A QSAR study was performed on percentage of inhibition of the corresponding compounds using multiple linear regressions. The predictive ability of the obtained model was verified by cross-validation and chance correlation. The final model showed that the calculated and predicted activities are in good agreement with their observed antituberculosis activities (R (cross-validation) = 0.87).

Results and major conclusion: Results of the biological assay showed that three compounds (**8c, 9a, 10b**) were antimycobacterial agents showing MIC value of 6.25 µg.ml^{-1}.It was also concluded that all three active compounds belong to nitroimidazoles and sulfonyl compound **9a**, was the most active analogue. The results of QSAR study demonstrated that electronic distribution is among the most important determining factors for activity in this series of compounds.

Keywords: Synthesis, antituberculosis activity, QSAR study, 1,3,4-thiadiazoles

INTRODUCTION

Mycobacterium tuberculosis, the causative organism for tuberculosis (TB), claims many human lives each year (1). Among the infected individuals, approximately eight million show active TB of which almost two million die from the disease (2).

Exponential increase in TB cases has been greatest in areas with high prevalence of HIV infections (3). The association of TB and HIV infection is so dramatic that in some cases, nearly two-thirds of the patients diagnosed with TB are also HIV seropositive (4).

Three major approaches have been employed to control TB; the sanitarium with fresh air and nutrition diet; vaccination; and chemotherapy (2).

A number of agents including para- amino salicylic acid (PAS), isoniazide (INH), rifampicin (RMP), pyrazinamide (PZA) and cycloserine have been used against TB (2). However, new antitubercular drugs with new mechanisms of action have not been developed in the last thirty

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years (5). Furthermore, the increase in drug resistant *Mycobacterium tuberculosis* isolates during recent years presents therapeutic challenges to physicians in selection of antimicrobial agents. Thus, development of new agents with potent antituberculosis activities and fewer adverse effects is urgently desired (6). The use of 5-nitroheterocycles as antibacterial, antiprotozoal and anticancer agents is well established and remains as an area of active research. Recently, two series of 5-nitrofuranyl amides with potent antituberculosis activity have been reported (7,8). Furthermore, PA-824 **1** (Figure 1), a bicyclic nitroimidazole, originally investigated as radiosensitizer for use in cancer chemotherapy, was found to possess activity against cultured replicating *M. tuberculosis* (5). In addition the 1,3,4-thiadiazole ring system is known to have several biological and antibacterial properties (9-10).
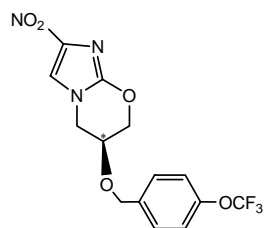

Figure 1. PA-824(**1**), a bicyclic nitroimidazole having antitubercular activity.

Based on these findings, synthesis, antimycobacterial activity and QSAR study of some new hybrid compounds using a 5-nitrohetrocycle and 1,3,4-thiadiazole ring as antitubercular agents (10-12), and also synthesis of 2-(nitroaryl)-5-(nitrobenzylthio)-1,3,4-thiadiazole have previously been reported (13-14).

In continuation of research for new antitubercular agents, in this study some 2-(nitroaryl)-5-(nitrobenzylsulfinyl and sulfonyl)-1,3,4-thiadiazole derivatives have been synthesiaed and evaluated for antituberculosis activity. Subsequently, a QSAR analysis was performed to obtain a deeper knowledge about the structural requirement for activity in this series of compounds.

CHEMISTRY

Synthesis of compounds **2-5** were according to the pathway illustrated in Scheme 1. Reaction of nitroaryl aldehyde **2** with thiosemicarbazide in

refluxing ethanol afforded compound **3**. The 2-amino-5-(nitroaryl)-1,3,4-thiadiazole **4** was prepared by oxidative cyclization of the corresponding 5-nitroaryl carboxaldehyde thio-semicarbazone **3**(9). Diazotization of **3** in hydrochloric acid in the presence of copper powder gave 2-chloro-5-(nitroaryl)-1,3,4-thia-diazole **5.** Reaction of 5 with thiourea in refluxing ethanol afforded 2-mercapto-5-(nitroaryl)-1,3,4-thiadiazoles **6** (9).

The procedure for the synthesis of 2-(nitroaryl)-5-(nitrobenzylthio)-1,3,4-thiadiazol **7** had been reported in our previous papers (13-14).

While, synthesis of 2-(nitroaryl)-5-(nitrobenzyl-sulfinyl)-1,3,4-thiadiazoles (**8a-f**) were performed through oxidation of thio analogues with H_2O_2 at 2-8 °C, oxidation with excess of H_2O_2 at 60°C, was used for preparation of 2-(nitroaryl)-5-(nitrobenzylsulfonyl)-1,3,4-thia-diazoles (**9a-f**).

Purities of the synthesized compounds were checked by TLC on silica gel. The IR, H-NMR, Mass Spectroscopy were used to confirm the structure of the synthesized compounds.

According to NMR spectra, the peaks relating to geminal protons of CH_2 group in nitrobenzyl-sulfinyl derivatives **8a-c** was observed as two doublets (J= 13 Hz), this effect was due to vicinity of the chiral sulfoxide center.

MATERIALS AND METHODS

All chemicals were obtained from Sigma and Merck chemical companies. TLC analyses were performed on a 3-10 cm aluminium sheet precoated with silica gel 60-254 (Merck). Melting points were taken on a Kofler hot stage apparatus and are uncorrected. IR spectra (KBr) were recorded on Shimadzu IR spectrophoto-meter. The mass spectra were run on a Finigan TSQ-70 spectrometer (Finigan, USA) at 70 eV. ^{1}H NMR (80 MHz) spectra were taken using CDCl$_3$ as solvent and tetramethylsilane (TMS) as internal standard.

Synthesis of the intermediate compounds (**2-6**) was performed according to the reported method (13, 14), and is shown in Scheme 1.

Synthesis of 2-(nitroaryl)-5-(nitrobenzylthio)-1,3,4-thiadiazoles **7a-f**

To a mixture of 2-(nitroaryl)-5-mercapto-1,3,4-thiadiazole (1mmol) and nitrobenzylchloride (1mmol) in ethanol (15ml) was added, 5 ml of KOH soloution (85%) dropwise and the mixture
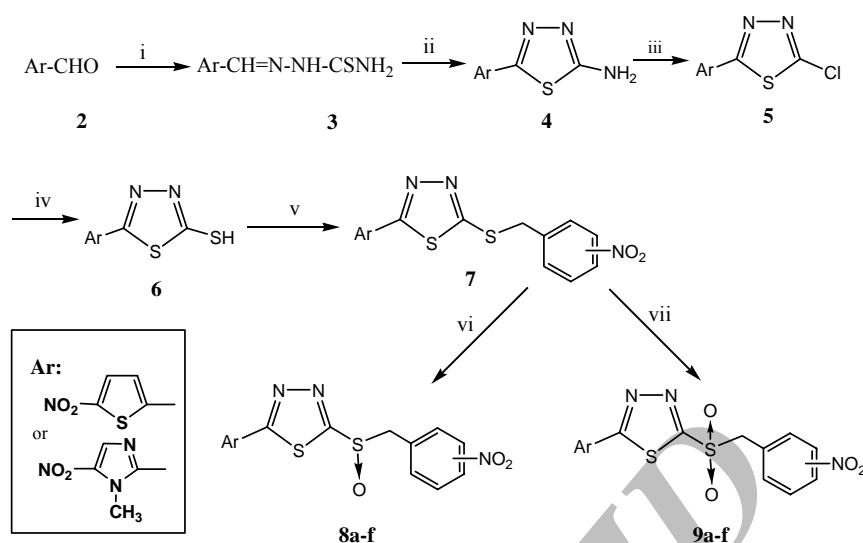
Scheme 1. Synthesis of compounds **8a**-f and **9a-f.** Reagents and conditions; (i) thiosemicarbazide, EtOH, HCl, reflux; (ii)ammonium ferric sulfate, H₂O, reflux; (iii) NaNO₂, HCl, Cu; (iv) thiourea, EtOH, reflux; (v) nitrobenzyl chloride, NaOH, EtOH, rt ; (vi) H₂O₂, CH₃COOH, 2-8 ºC; (vii) H₂O₂, CH₃COOH, 60 ºC.

was stirred overnight at room temperature. Water was added and the separated solid was filtered, washed with water and crystallized from ethanol-water.

General procedure for preparation of 2-(nitroaryl)-5-(nitrobenzylsulfinyl)-1,3,4-thiadiazoles **8a-f**

To a stirring mixture of 2-(nitroaryl)-5-(nitro-benzylthio)-1,3,4-thiadiazole (1mmol) in glacial acetic acid (3ml) was added 30% H₂O₂ (3 ml) and the mixture was stirred at 2-8 ºC for 48 hours. Water was then added and the precipitate was filtered and purified by column chromatography eluting with 5% EtOH-CHCl₃.

2-(2-nitrobenzylsulfinyl)-5-(1-methyl-5-nitro-1H-imidazol-2-yl)-1,3,4-thiadiazole **8a**

was prepared from oxidation of 2-(2-nitro-benzyl-thio) -5 -(1-methyl- 5- nitro-2- imidazolyl)-1,3,4-thiadiazole (**7a,** 1mmol, 394mg)

yield: 53%; mp 165-166 º C; IR (KBr) (cm⁻¹): 1526,1337 (NO₂), 1060(SO), ¹HNMR (CDCl₃, 500 MHz, δ ppm): 8.17 (d, 1H, H₃-phenyl, J=8 Hz), 8.11 (s,1H, H₄-imiazole), 7.65-7.59 (m, 2H, H₄,H₅-phenyl), 7.36 (d, 1H, H₆-phenyl, J= 8 Hz), 5.35(d, 1H, S-CH₂, J=13Hz), 4.74 (d,1H, S-CH₂, J= 13 Hz), 4.61 (s, 3H, N-CH₃). MS (m/z %): 394 (M⁺,10), 330 (10), 242 (10), 170 (31), 133(100).

2-(3-nitrobenzylsulfinyl)-5-(1-methyl-5-nitro-1H-imidazol-2-yl)-1,3,4-thiadiazole **8b**

was prepared from oxidation of 2-(3-nitro-benzylthio)-5- (1-methyl-5-nitro-2-imidazol-yl)-1,3,4- thiadiazole (**7b**,1mmol,394mg)

yield: 42%, mp 175-176 ºC; IR (KBr) (cm⁻¹): 1542,1325 (NO₂), 1055 (SO); ¹HNMR (CDCl₃, 500 MHz, δ ppm): 8.24 (d, 1H, H₄-phenyl, J=8Hz), 8.08 (s, 1H, H₄-imidazoe), 7.95 (s, 1H, H₂-phenyl), 7.60-7.54 (m, 1H, H₅,H₆-phenyl), 4.72 (d, 1H, S-CH₂, J=13.4 Hz), 4.61 (s, 3H, N-CH₃) and 4.50 (d, 1H, S-CH₂, J= 13.4 Hz); MS (m/z %): 394 (M⁺,10), 332 (10), 243 (10), 136 (90), 78 (100), 65 (20).

2-(4-nitrobenzylsulfinyl)-5-(1-methyl-5-nitro-1H-imidazol-2-yl)-1,3,4-thiadiazole **8c**

was prepared from oxidation of 2-(4-nitrobenzyl-thio)-5-(1-methyl-5-nitro-2-imidazolyl)-1,3,4-thiadiazole (**7c**,1mmol, 394mg)

yield: 47%; mp 198-199 ºC; IR (KBr) (cm⁻¹): 1544, 1340 (NO₂), 1055 (SO). ¹HNMR (CDCl₃, 500 MHz, δ ppm): 8.20 (d, 2H, phenyl, J=8 Hz), 8.12 (s, 1H, H₄-imidazoe), 7.39 (d, 2H, phenyl, J=8 Hz), 4.73 (d, 1H, S-CH₂, J= 13 Hz), 4.65 (s, 3H, CH₃), 4.61 (d, 1H, S-CH₂, J= 13 Hz); MS (m/z %): 394 (M⁺,30), 378 (15), 243 (10), 193 (25),136 (100), 106 (90), 78 (95).

2-(2-nitrobenzylsulfinyl)-5-(5-nitrofuran-2-yl)-1,3,4-thiadiazole **8d**

was prepared from oxidation of 2-(2-nitro-benzylthio)-5- (5-nitro-2-furyl)- 1,3,4- thiadiazole (**7d**, 1mmol, 379 mg)

yield: 53%, mp 155-156 °C l; IR (KBr)(cm^{-1}): 1523, 1340 (NO$_2$), 1055 (SO);

HNMR (CDCl$_3$, 500 MHz, δ ppm): 8.19-8.17 (m, 1H, H$_3$-phenyl), 7.63-7.61(m, 2H, H$_4$, H$_5$-phenyl), 7.50 (d, 1H, H$_4$-furyl, J= 4Hz), 7.47 (d, 1H, H$_3$-furyl, J= 4 Hz), 7.37-7.31(m, 1H, H$_6$-phenyl), 5.39 (d, 1H, S-CH$_2$, J=13Hz) and 4.72 (d, 1H, S-CH$_2$, J= 13 Hz).

2-(3-nitrobenzylsulfinyl)-5-(5-nitrofuran-2-yl)-1,3,4-thiadiazole **8e**

was prepared from oxidation of 2-(3-nitrobenzylthio)-5- (5-nitro-2-furyl)- 1,3,4-thiadiazole (**7e**, 1mmol,379 mg)

yield: 48%, mp 163-164 ºC IR (KBr); ν$_{max}$ (cm^{-1}): 1526, 1344 (NO$_2$), 1055 (SO), HNMR (CDCl$_3$, 500 MHz, δ ppm) 8.26-8.23 (m, 1H, H$_4$-phenyl), 8.02-8.00 (m, 1H, H$_2$- phenyl), 7.59-7.55 (m, 2H, H$_2$, H$_5$, H6-phenyl), 7.50 (d, 1H, H$_4$-furyl, J=4Hz), 7.45 (d, 1H, H$_3$-furyl, J=4Hz), 4.72 (d, 1H, S-CH$_2$, J=13.5Hz) and 4.55 (d, 1H, S-CH$_2$, J=13 Hz). MS (m/z %) 380 (M$^+$, 40), 351 (54), 300 (11), 285 (36), 135 (100).

2-(4-nitrobenzylsulfinyl)-5-(5-nitrofuran-2-yl)-1,3,4-thiadiazole **8f**

was prepared from oxidation of 2-(4-nitrobenzylthio)-5- (5-nitro-2-furyl)- 1,3,4-thiadiazole (**7f**,1mmol, 379 mg)

yield: 41%: mp 184-185 ºC; IR (KBr) (cm^{-1}): 1507, 1344 (NO$_2$),1065 (SO), HNMR (CDCl$_3$, 500 MHz, δ ppm) 8.19 (d, 2H, H$_3$, H$_5$-phenyl, J= 8 Hz), 7.49 (d, 1H, H$_4$- furyl, J=4 Hz), 7.44 (d, 1H, H$_3$-furyl, J=4 Hz), 7.36 (d, 2H, H$_2$, H$_6$-phenyl, J= 8 Hz), 4.71 (d, 1H, S-CH$_2$, J=13 Hz) and 4.55 (d, 1H, S-CH$_2$, J=13 Hz); MS (m/z %) 380 (M$^+$, 13), 351 (10), 298 (5), 136 (90), 105 (45), 88(86), 77(100).

General procedure for preparation of 2-(nitroaryl)-5-(nitrobenzylsulfonyl)-1,3,4-thiadiazoles **9a-f**

To a stirring mixture of 2-(nitroaryl)-5-(nitrobenzylthio)- 1,3,4- thiadiazole (1mmol) in glacial acetic acid (3ml) was added 3 ml of 30%

H$_2$O$_2$ and the mixture was stirred at 60°C for 20 minutes. After cooling, water was added; the precipitate was filtered and recrystallized from ethanol.

2-(2-nitrobenzylsulfonyl)-5-(1-methyl-5-nitro-1H-imidazol-2-yl)-1,3,4-thiadiazole **9a**

Was prepapred from oxidation of 2-(2-nitrobenzylthio)-5- (1-methyl-5-nitro-2-imidazolyl)- 1,3,4- thiadiazole (**7a**, 1mmol, 410 mg)

yield: 85%, mp 189-190 ° C; IR (KBr) (cm^{-1}): 1544, 1347 (NO$_2$), 1335 (SO$_2$), ^{1}HNMR (CDCl$_3$, 80 MHz, δ ppm): 8.14 (d, 1H, H$_3$-phenyl), 8.12 (s, 1H, H$_4$-imidazole), 7.60-7.73 (m, 3H, phenyl), 5.46 (s, 2H, CH$_2$) and 4.62 (s, 3H, N-CH$_3$); MS (m/z %): 410 (M$^+$,10), 344 (20), 329 (8), 136 (90), 88(100), 67(13).

2-(3-nitrobenzylsulfonyl)-5-(1-methyl-5-nitro-1H-imidazol-2-yl)-1,3,4-thiadiazole **9b**

was prepared from oxidation of 2-(3-nitrobenzylthio)-5- (1-methyl-5-nitro-2-imidazolyl)- 1,3,4- thiadiazole (**7b**, 1mmol, 410 mg)

yield: 55%; mp 214-215 ºC ; IR (KBr) (cm^{-1}): 1526, 1350 (NO$_2$), 1337, 1161(SO$_2$), ^{1}HNMR (CDCl$_3$, 500 MHz, δ ppm): 8.29 (d, 1H, H$_2$-phenyl, J= 8 Hz), 8.22 (s, 1H, H$_4$-phenyl), 8.11 (s, 1H, H$_4$-imidazole), 7.80 (d, 1H, H$_6$-phenyl, J=8Hz), 7.63(t, 1H, H$_5$-phenyl, J= 8Hz), 4.99 (s, 2H, S-CH$_2$), 4.64 (s, 3H, N-CH$_3$). MS (m/z %): 410 (M$^+$, 27), 345 (68), 329 (45), 298 (31), 288 (13), 133 (100).

2-(4-nitrobenzylsulfonyl)-5-(1-methyl-5-nitro-1H-imidazol-2-yl)-1,3,4-thiadiazole **9c**

was prepared from oxidation of 2-(4-nitro-benzylthio)-5-(1-methyl-5-nitro-2-imidazol-yl)-1,3, 4- thiadiazole (**7c,** 1mmol, 410 mg)

yield: 86%; mp 230-231°C; IR (KBr) (cm^{-1}): 1520, 1340 (NO$_2$), 1370, 1150 (SO$_2$) HNMR (CDCl$_3$, 500 MHz, δ ppm): 8.26 (d, 2H, H$_3$, H$_5$-phenyl, J= 8 Hz), 8.11 (s, 1H, H$_4$-imidazole), 7.61 (d, 2H, H$_2$, H$_6$-phenyl, J= 8 Hz), 4.99 (s, 2H, S-CH$_2$) and 4.69 (s, 3H, N-CH$_3$); MS (m/z %) 410 (M$^+$, 10), 345 (13), 136 (100), 89 (54), 63 (13).

2-(2-nitrobenzylsulfonyl)-5-(5-nitrofuran-2-yl)-1,3,4-thiadiazole **9d**

was prepared from oxidation of 2-(2-nitrobenzylthio)-5- (5-nitro-2-furyl)- 1,3,4-thiadiazole (**7d**,1 mmol, 395 mg)

yield 82%; mp 162-163 ºC, IR (KBr) (cm^{-1}) 1513, 1340 (NO$_2$), 1150(SO$_2$). ^{1}HNMR (CDCl$_3$, 500 MHz, δ ppm): 8.15 (d, 1H, H$_3$-phenyl, J=8 Hz), 7.72-7.64 (m, 2H, H$_4$, H$_5$-phenyl), 7.60 (d, 1H, H$_6$-phenyl, J=8 Hz), 7.55 (d, 1H, H$_4$-furyl, J= 4Hz), 7.51(d, 1H, H$_3$-furyl, J= 4Hz), 5.46 (s, 2H, CH$_2$); MS (m/z %): 396 (M$^+$,12), 393 (25), 377 (30), 350 (100).

2-(3-nitrobenzylsulfonyl)-5-(5-nitrofuran-2-yl)-1,3,4-thiadiazole **9e**

was prepared from oxidation of 2-(3-nitrobenzylthio)-5- (5-nitro-2-furyl)- 1,3,4-thiadiazole (**7e,** 1mmol, 395 mg)

yield: 85%; mp 175-176 ºC; IR (KBr) (cm^{-1}) 1532, 1347 (NO$_2$), 1150(SO$_2$); ^{1}HNMR (CDCl$_3$, 500 MHz, δ ppm): 8.29 (d, 1H, H$_4$-phenyl, J=8Hz), 8.24 (s, 1H, H$_2$-phenyl), 7.77 (d, 1H, H$_6$-phenyl, J=8Hz), 7.62 (t, 1H, H$_5$-phenyl, J=8Hz), 7.54(d, 1H, H$_4$-furyl, J=3.8Hz), 7.51(d, 1H, H$_4$-furyl, J=4Hz), 4.99 (s, 2H,S-CH$_2$). MS (m/z %): 396 (M$^+$, 13), 371 (37), 377 (30), 315 (18), 136(100), 97.4(20)

2-(4-nitrobenzylsulfonyl)-5-(5-nitrofuran-2-yl)-1,3,4-thiadiazole **9f**

was prepared from oxidation of 2-(4-nitrobenzylthio)-5- (5-nitro-2-furyl)- 1,3,4-thiadiazole (**7f** , 1mmol, 395 mg)

yield 53%; mp 232-233 º C, IR (KBr) (cm^{-1}) 1530, 1345 (NO$_2$), 1150(SO$_2$); 1HNMR(CDCl$_3$, 80 MHz, δ ppm) 8.25 (d, 2H, H$_3$,H$_5$-phenyl, J=8Hz), 7.64 (d, 2H, H$_2$, H$_6$- phenyl, J=8Hz), 7.53 (d, 1H, H-furyl, J= 4Hz), 7.50 (d, 1H, H-furyl, J= 4 Hz), 5.47 (s, 2H, CH2); MS (m/z %): 396 (M$^+$, 12), 380 (15), 331 (45), 156 (8), 133(100)

Biological assay

The compounds were screened for antituberculosis activity under direction of the US National Institute of Health, NIAID division. All compounds were initially screened against *Mycobacterium tuberculosis* strain H$_{37}$Rv at single concentration of 6.25 µg.ml^{-1} in BACTEC 12B medium using a broth microdilution assay. Compounds demonstrating growth inhibition ≥ 90% in the primary screening were considered active.

The active compounds were re-tested by serial dilution beginning at the concentration of 6.25µg ml^{-1} against *M. tuberculosis* H$_{37}$Rv to determine the actual minimum inhibitory concentration (MIC) in the BACTEC 460 radiometric system and BACTEC 12B medium.

The MIC is defined as the lowest concentration reducing fluorescence to 90% of controls. The significance of this value depends on several factors such as compound structure, novelty, toxicity, and potential mechanism of action. INH (MIC= 0.025- 0.05 µg ml^{-1}) and RMP (0.025-0.125 µg ml^{-1}) were used as positive control drugs. (15) (Table1)

QSAR analysis

In an attempt to determine the role of structural features that appear to influence the observed activity of the reported compounds, QSAR studies were undertaken using the multiple linear regression (MLR) method. Several physicochemical descriptors such as hydrophobicity, topological indices, electronic parameters and steric factors are usually used in QSAR studies in order to find the effects of different structural properties on the biological activity of compound of interest.

Since the calculated values of some electronic descriptors depend on the three-dimensional molecular geometry, the optimum 3-D geometry of the molecules were obtained by Hyperchem software (Hypercube Inc, USA), using AM1 semi-empirical method. Due to presence of a large number of atoms in the studied molecules (at least 20 carbon and hetroatoms) semi-empirical methods were preferred over the ab initio method to save calculated time (16). The resulting structures were used to calculate constitutional, functional and topological descriptors by Dragon software. Meanwhile some electronic descriptors such as frontier molecular orbital (HOMO, LUMO), dipole moment and partial charges were calculated by the Hyperchem software. The calculated descriptors were used as independent variables in the regression analysis. The dependent variable is the activity of compounds expressed as the logarithm of reciprocal inhibition percentage [log (1/k$_i$)] (17).

Table 1. In vitro antituberculosis activity of compounds **8a-f** and **9a-f**
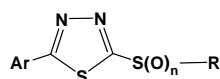
Comp.	Ar	n	R	Inhibition (%)	MIC(μg/ml)
8a	1-methyl-5-nitroimidazol-2-yl	1	2-nitrobenzyl	49	>6.25
8b	1-methyl-5-nitroimidazole-2yl	1	3-nitrobenzyl	35	>6.25
8c	1-methyl-5-nitroimidazole-2yl	1	4-nitrobenzyl	99	6.25
9a	1-methyl-5-nitroimidazole-2-yl	2	2-nitrobenzyl	100	6.25
9b	1-methyl-5-nitroimidazole-2-yl	2	3-nitrobenzyl	90	6.25
9c	1-methyl-5-nitroimidazole-2-yl	2	4-nitrobenzyl	88	>6.25
8d	5-nitro-2-furyl	1	2-nitrobenzyl	50	>6.25
8e	5-nitro-2-furyl	1	3-nitrobenzyl	33	>6.25
8f	5-nitro-2-furyl	1	4-nitrobenzyl	24	>6.25
9d	5-nitro-2-furyl	2	2-nitrobenzyl	26	>6.25
9e	5-nitro-2-furyl	2	3-nitrobenzyl	15	>6.25
9f	5-nitro-2-furyl	2	4-nitrobenzyl	79	>6.25
Isoniazide					0.05
Rifampicin					0.125

Table 2.Calculated and experimental data for the synthesized compounds **8a-f** and **9a-f**

	Descriptors matrix		Activity vector (log(1/ki))	
Compound	Mp	MPC	Experimental	Predicted by Cross validation
8a	0.73	0.32	-1.6	-1.64
8b	0.73	0.22	-1.54	-1.41
8c	0.73	0.39	-1.9	-1.71
9a	0.72	0.34	-2	-2.02
9b	0.72	0.34	-1.95	-2.04
9c	0.72	0.35	-1.94	-2.05
8d	0.74	0.39	-1.84	-1.44
8e	0.74	0.39	-1.51	-1.41
8f	0.74	0.32	-1.38	-1.32
9d	0.74	0.43	-1.41	-1.54
9e	0.74	0.35	-1.17	-1.43
9f	0.74	0.48	-1.89	-1.57

[*]Mp= molecular polarizability; MPC= Maximum Positive Charge

The presence of co-linear descriptors may cause difficulties in certain aspects of forming a QSAR model, and hence the predictivity and generalization of a model will be generally failed in the presences of highly co-linear descriptors. In order to overcome this problem, the correlation of the calculated descriptors with each other was examined and collinear descriptors ($r>0.9$) were determined. Among these, one of them, which had the highest correlation with the dependent variable, was retained and others were removed from the data matrix.

The resulted matrix of non co-linear descriptors were used to find a multilinear equation of the form $\log(1/k_i)=b_0+b_1DES_1+b_2DES_2+.....$between activity and structural parameters. The stepwise selection and elimination of variables by SPSS software was used to find the best set of descriptors (18-20).

To improve the regression coefficients and attain a more predictive equation, **8d** and **9f** were considered as outliers and excluded from the model.

Significant correlations were found in model using constitutional and electronic descriptors (Equations 1 and 2, respectively):

$Log(1/ki)=-23.6(\pm4.19)+30.11(\pm5.27)$ Mp   (1)
N= 10 R^2= 0.77   Se= 0.15   F= 27.45

$Log(1/ki)=24.88(\pm3.718)+32.464(\pm5.168)Mp-1.49(\pm.0796)MPC$ (2)
N= 10 R^2= 0.85   Se= 0.13 R^2_{cv} =0.74   F= 19.77

In these equations, the values in the parentheses represent the standard deviation of the coefficients. N, R, Se and F are numbers of components, correlation coefficient, standard error of regression and Fisher's F-ration, respectively. There are different validation techniques to simulate the predictive ability of a model. Cross validation is the most common validation technique where a number of modified data sets are created by deleting, one or a small number of compounds (in such a way that each object is removed once from the original data set). For each reduced data set, the model is calculated and responses for the deleted compounds are predicted from the model (20-21). In this study, the predictive power of the models was checked by leave one out cross-validation and the square of the cross-validated correlation coefficient (R^2_{cv}) was used to measure the models predictivity (18-20). For examples, the predicted activities refined by the calibration data and those obtained by the cross-validation procedure are listed in Table 2 for Equation 2 and are plotted against the experimental activity in Figure 2. Since, exclusion of the two compounds (**8d** and **9f**) could dramatically increase R^2_{cv} from 0.56 to 0.74; these two compounds were not considered in the final equation. The results showed that the calculated and predicted activities for the rest of compounds were in good agreement with the observed activities (R^2= 0.85, R^2_{cv}=0.74).

Y-scrambling or response permutation testing is another widely used technique to check the robustness of a QSAR model, and to identify models based on chance correlation. The test was performed by calculating the quality of the model (R^2) while modifying the sequence of the response vector (activity), randomly (22). The original model had no significant chance correlation, since a large difference could be observed in the quality of the original model and those associated with a

model obtained with random responses ($\sum R^2$ /n= 0.35). This procedure was repeated one hundred times (n= 100).

RESULTS AND DISCUSSION

Results of the biological assay showed that, all three active compounds (**8c, 9a, 9b**) belong to nitroimidazoles and sulfonyl compound **9a**, was the most active analogue.

The final QSAR model described that the antitubercular activity for the compounds used in this study are affected by:

1)   Mean atomic polarizability (Mp) which is a constitutional descriptor, and is measured by summation of the atomic contributions ($\sum a_iE_i$, where E_i is the electric field at the corresponding atom and a_i the corresponding polarizability assumed to be isotropic) (16).

2)   Maximum Positive Charge, among electronic descriptors, was calculated for thiadiazole derivatives. The importance of this parameter in describing the activity of a different set of thiadiazole analogues has previously been reported (11).

Maximum positive charge represents the maximum positive charge of atoms in a molecule (Q_{max}= max (q^+) and the charge polarization is the mean absolute atomic charge in molecules defined as $P=\sum (|q|/A) = Q/A$

The presence of molecule polarizability in EQ and its direct relationship with activity vector was in accord with results obtained from structure activity relationship (SAR)
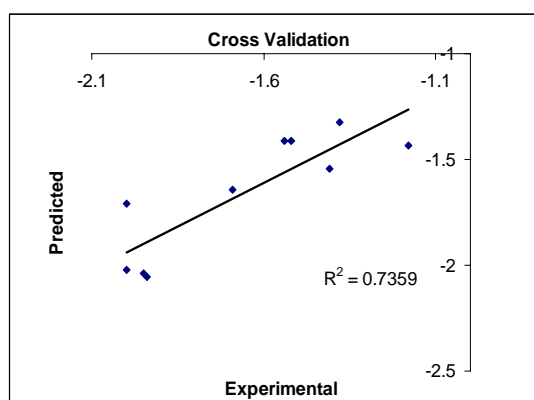

Figure2. Plot of Predicted activity against experimental activity for compounds **8a-f** and **9a-f** (R^2 = 0.736)

It clearly showed that the compounds containing nitroimidazole are more potent than their corresponding nitrofuryl series which could be

due to lower molecular polarizability values. Addition of MPC with a negative coefficient to second equation increased the correlation of the resulting equation. Since polarization can be related to two main contributios of electronic and atomic polarization, it seems that polarization, rather than electrostatic field are affecting the positive and negative charge these two descriptors.

CONCLUSION

In continuation of our previous studies to find new agents against *Mycobacterium tuberculosis* a series of 2-(nitroaryl)-5-(nitrobenzylsulfinyl and sulfonyl)-1,3,4-thiadiazole were synthesized and their antituberculosis activity were determined.

In QSAR study, different set of molecular descriptors were calculated to predict the antituberculosis activity of the twelve synthesized compounds by MLR and the importance of constitutional descriptors represented by molecular polarizabilty and electronic descriptors represented by most positive charge was demonstrated by equation 2. Except in the case of two derivatives **8d** and **9f**, activity of all compounds were predicted with good approximation (R^2= 0.85, R^2_{cv}=0.74). From the results of QSAR study, it is concluded that electronic distribution is among the most important factors in determination of activity for this group of compounds.

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