Academic Sciences Asian Journal of Pharmaceutical and Clinical Research

Vol 5, Suppl 3, 2012 ISSN - 0974-2441

Research Article

SYNTHESIS, EVALUATION AND QSAR ANALYSIS OF 5-NITROFURAN-2-YL/4-NITROPHENYL METHYLENE SUBSTITUTED HYDRAZIDES AS ANTITUBERCULAR AGENTS

REVATHI A. GUPTA*, SATISH G. KASKHEDIKAR

Medicinal Chemistry Laboratory, Department of Pharmacy, Shri G. S. Institute of Technology and Science, 23 Park Road, Indore 452003, M.P., India. Email: arunrevathi19@gmail.com

Received:29 July 2012, Revised and Accepted: 10 August 2012

ABSTRACT

A series of novel *N*'-((5-nitrofuran-2-yl/4-nitrophenyl) methylene) substituted hydrazides (3a-3i & 4a-4i) was synthesized, and tested for *in vitro* antimycobacterial activity, and their quantitative structure activity relationship (QSAR) of hydrazide derivatives is reported here. The results of anti-mycobacterial activity study indicated that the presence of halogen substitution at benzohydrazide moiety improved the activity of these derivatives. Preliminary structure activity relationship analysis revealed that 5-nitrofuran-2-yl-methylene analogs more potent than 4-nitrobenzylidene substituted analogs. QSAR model was developed correlating the antimycobacterial activity with the structural features of the compounds and the predictive capability of the models was estimated using validation parameters. The QSAR analysis results indicated the radial distribution function (RDF030v, RDF135m & RDF120u) might be helpful in describing the antimycobacterial activity of hydrazides.

Keywords: Antimycobacterial, Hydrazide derivatives, SQ-MLR, QSAR.

INTRODUCTION

Tuberculosis (TB) is presently regarded as the most dangerous infective disease world-wide and also a significant socio-economic burden in India[1]. At present, according to statistics, TB kills four people every minute somewhere in the world and accounts for about two million deaths per year. It is estimated that one-third of the world's population is currently infected with the TB bacillus and 30 million people will die in the next 10 years[2]. In addition, the simultaneous presence of HIV infection, the spread of drug-resistant strains of MTB,[3] the length and complexity of current TB treatment regimens results in poor patient compliance, a major contributing factor in the emergence of multi-drug-resistant tuberculosis[4] (MDRTB) and extensively drug-resistant tuberculosis (XDRTB)[5]. In spite of this, emergence of multiple drug resistant TB together with the spread of severe opportunistic disseminated infections that have focused the attention of scientific community throughout the world on urgent need for antimycobacterial agents. The development of antituberculosis drug resistance is mainly contributed by the higher lipid and mycolic acid content in the mycobacterial cell wall. In the process of development of new antitubercular agents, the main drug targets are cell wall biosynthesis, nucleic acid synthesis, and other pathways. Hence, the development of newer antimycobacterial agents is essential to overcome the rapidly developing drug resistance and side effects with high efficacy.

Hydrazones belong to the Schiff base family containing azomethine –NHN=CH– protons and they are considered as the important class of compounds for the development of new drugs[6]. Hydrazide-hydrazone derivatives have been claimed to possess antimicrobial[7]. antimycobacterial[8-10],anticancer,[11,12]anti-inflammatory,[13,14]antiviral[15] and antimalarial[16] activities. Further, careful literature survey for functional groups which could be considered as pharmacophores for the antitubercular activities revealed that the hydrazone moiety is common among most of the antitubercular agents[17,18]. So, considering this chemotherapeutic value, hydrazones were prompted us to synthesize some newer derivatives of Schiff bases as antimycobacterial agents.

Several in silico techniques are utilized in the process of drug design and development of antitubercular agents. One such technique is quantitative structure activity relationship (QSAR).Quantitative structure activity relationship has been traditionally perceived as means of establishing correlation between trends in chemical structure modification and respective changes of biological activity[19]. QSAR is widely used in development of antitubercular agents. This quantitative technology can be utilized to improve the structure of the inhibitor molecule and to interpret the improved structure in terms of favorable biological interactions[20]. Thus, the use of predictive computational (in silico) QSAR models allows the biological properties of virtual structures to be predicted, and a more informed choice of target to be selected for synthesis[21]. The use of computational approaches for the estimation of the activity of various molecules as drug candidates prior to their synthesis can save the resources and accelerate the drug discovery procedure[22]. We carried out QSAR analysis and established QSAR models to guide further structural optimization and predict the potency and physiochemical properties of clinical drug candidates.

From the aforementioned findings and with the aim of obtaining newer anti-mycobacterial compounds we hereby report the synthesis, antimycobacterial evaluation and QSAR studies of *N*'-((5-nitrofuran-2-yl/4-nitrophenyl)methylene) substituted hydrazide derivatives.

MATERIAL AND METHODS

All the chemicals used in the synthesis of compounds were of synthetic grade, and they were procured from Sigma, SD-fine, Himedia and E. Merck. The synthesis of the title compounds **3a-3i & 4a-4i** was carried out as depicted in Scheme 1. Thin layer chromatographic method was used for monitoring the progress of the reactions on silica gel sheets (Merck silica gel G) and purity of compound was ascertained by single spot TLC. The purification of intermediates and final compounds was carried out through recrystallization and flash column chromatography technique. For the purpose of chromatography glass column (high 18 inch with internal diameter 20mm), column grade silica gel mesh # 240-400 as stationary phase and appropriate solvent system as mobile phase were used. Melting points were determined by open tube capillary method and were uncorrected. ^{1}H nuclear magnetic resonance (^{1}H NMR) spectra were recorded on BRUKER 400MHz FT-NMR Spectrometer for solids - DSX- AV-III 400 using appropriate deuterated solvents and are expressed in parts per million (δ, ppm) downfield from tetramethylsilane (internal standard). Infrared (IR) spectra were recorded on Perkin Elmer FTIR spectrometer using KBr pellets. The mass spectroscopy was recorded on a LC-ESI-Q-TOF Micro Mass Spectrometer.

General procedure for the synthesis of substituted methyl esters (1)

Substituted benzoic acid (40 mmol) and dried methanol (50mL) was refluxed for 4-6 h in the presence of catalytic amount of concentrated sulfuric acid (2.0 mmol, 1.0mL) with constant stirring. The progress of the reaction was monitored through TLC. After completion of reaction, excess solvent was removed under reduced pressure and the concentrate was diluted with water and extracted with ether/ chloroform. The organic layer was washed with saturated $NaHCO_3$ solution, dried over anhydrous sodium sulphate

and evaporated. The obtained product was recrystallized from an appropriate solvent.

General procedure for synthesis of substituted benzohydrazide (2)

Method A: The substituted methyl ester (40 mmol) was added to 50–60°C pre-heated hydrazine hydrate 99% (v/v) (132 mmol) in round bottom flask. The reaction mixture was refluxed for 15-30 minutes. The reaction mixture was sequentially cooled in a water bath, followed by ice bath. The precipitate was filtered, washed with ice-cold water, dried and recrystallized in ethanol.

Method B: Substituted methyl ester (40 mmol) was dissolved in methanol (40 mL). Hydrazine hydrate (99% v/v) (60 mmol) was added drop wise to the solution and refluxed for 16-18 h. The progress of the reaction was monitored through TLC. After completion of reaction, the mixture was cooled to rt and residue was poured into ice cold water. The precipitate was filtered, washed with ice-cold water, dried and recrystallized in ethanol.

General procedure for synthesis of hydrazones (3a-3i & 4a-4i)

A mixture of (25 mmol) substituted carboxylic acid hydrazide and appropriate aromatic aldehyde (25 mmol) was refluxed in ethanol (50 mL) in the presence of a catalytic amount of glacial acetic acid for about 4-7 h. The progress of the reaction was monitored through TLC. After completion of reaction, mixture was cooled to rt. The precipitates obtained was filtered off, washed with ethanol, dried and purified by chromatography to afford the corresponding hydrazone derivatives. Structures of the synthesized compounds were confirmed on the basis of spectral data.

N'-((5-nitrofuran-2-yl)methylene)benzohydrazide **3a.** M.p. 203-204°C; Yield - 80%; ^{1}H NMR (400 MHz, DMSO) δ in ppm: 12.10 (s, 1H, NH), 8.54 (s, 1H, =CH-Ar), 8.13-8.06 (m, 2H, Ar-H), 7.99-7.95 (m, 2H, Ar-H), 7.63-7.52 (m, 3H, Ar-H); IR (cm^{-1}) ν_{max} 3210 (NH), 1664 (C=O), 1596 (Ar-C=C), 1517 (*asym*, Ar-NO_2), 1323 (*sym*, Ar-NO_2); ESI-MS *m/z* : 282 (M+Na$^+$).

2-bromo-N'-((5-nitrofuran-2-yl)methylene)benzohydrazide **3b.** M.p. 206-208°C; Yield - 82%; ^{1}H NMR (400 MHz, DMSO) δ in ppm: 12.28 (s, 1H, NH), 8.67 (s, 1H, =CH-Ar), 8.14-8.12 (s, 1H, Ar-H), 7.87-7.75 (m, 4H, Ar-H), 7.60-7.59 (s, 1H, Ar-H); IR (cm^{-1}) ν_{max} 3421(NH), 1663 (C=O), 1595 (Ar-C=C), 1519 (*asym*, Ar-NO_2), 1319 (*sym*, Ar-NO_2), 1246 (C-O-C); ESI-MS *m/z* : 360 (M+Na$^+$).

3-bromo-N'-((5-nitrofuran-2-yl)methylene)benzohydrazide **3c.** M.p. 200-201°C; Yield - 84%; ^{1}H NMR (400 MHz, DMSO) δ in ppm: 12.28 (s, 1H, NH), 8.65 (s, 1H, =CH-Ar), 8.11-8.07 (d, 2H, Ar-H), 7.91-7.79 (m, 2H, Ar-H), 7.58-7.48 (m, 2H, Ar-H); IR (cm^{-1}) ν_{max} 3136 (N-H), 2998 (C-H) 1644 (C=O), 1557 (Ar-C=C), 1509 (*asym*, Ar-NO_2), 1349 (*sym*, Ar-NO_2), 1254 (C-O-C); ESI-MS *m/z* : 360 (M+Na$^+$).

4-bromo-N'-((5-nitrofuran-2-yl)methylene)benzohydrazide **3d.** M.p. 224-226°C; Yield - 85%; ^{1}H NMR (400 MHz, DMSO) δ in ppm: 12.28 (s, 1H, NH), 8.50 (s, 1H, =CH-Ar), 8.21-8.12 (d, 1H, Ar-H), 7.59-7.39 (m, 5H, Ar-H); IR (cm^{-1}) ν_{max} 2995 (C-H), 1651 (C=O), 1558 (Ar-C=C), 1509 (*asym*, Ar-NO_2), 1340 (*sym*, Ar-NO_2), 1254 (C-O-C); ESI-MS *m/z* : 360 (M+Na$^+$).

2,4-dichloro-N'-((5-nitrofuran-2-yl)methylene)benzohydrazide **3e.** M.p. 210-211°C; Yield - 87%; ^{1}H NMR (400 MHz, DMSO) δ in ppm: 12.31 (s, 1H, NH), 8.49 (s, 1H, =CH-Ar), 8.12-8.10 (d, 1H, Ar-H), 8.03-8.02 (d, 1H, Ar-H), 7.64-7.54 (m, 3H, Ar-H); IR (cm^{-1}) ν_{max} 3195 (N-H), 3018 (C-H), 1678 (C=O), 1560 (Ar-C=C), 1507 (*asym*, Ar-NO_2), 1349 (*sym*, Ar-NO_2), 1250 (C-O-C); ESI-MS *m/z* : 350 (M+Na$^+$).

4-methoxy-N'-((5-nitrofuran-2-yl)methylene)benzohydrazide **3f.** M.p. >270°C; Yield - 86%; ^{1}H NMR (400 MHz, DMSO) δ in ppm: 12.09 (s, 1H, NH), 8.38 (s, 1H, =CH-Ar), 7.79-7.90 (d, 2H, Ar-H), 7.79-7.78 (d, 1H, Ar-H), 7.74-7.23 (d, 1H, Ar-H), 7.09-7.05 (d, 2H, Ar-H), 3.84 (s, 3H, -OCH_3); IR (cm^{-1}) ν_{max} 3285(N-H), 3138 (C-H), 1659 (C=O), 1601,1507 (Ar-C=C), 1550 (*asym*, Ar-NO_2), 1353 (*sym*, Ar-NO_2), 1240 (C-O-C); ESI-MS *m/z* : 312 (M+Na$^+$).

4-methyl-N'-((5-nitrofuran-2-yl)methylene)benzohydrazide **3g.** M.p. 238-240°C; Yield - 81%; ^{1}H NMR (400 MHz, DMSO) δ in ppm: 12.10 (s, 1H, NH), 8.40 (s, 1H, =CH-Ar), 7.83-7.81(d, 2H, Ar-H), 7.79-7.78 (d, 1H, Ar-H), 7.36-7.34 (d, 2H, Ar-H), 7.26-7.25 (d, 1H, Ar-H), 2.38 (s, 3H, -CH_3); IR (cm^{-1}) ν_{max} 3395 (N-H), 2966 (C-H) 1663 (C=O), 1501 (Ar-C=C), 1560 (*asym*, Ar-NO_2), 1346 (*sym*, Ar-NO_2), 1263 (C-O-C); ESI-MS *m/z* : 296 (M+Na$^+$).

3,4,5-trimethoxy-N'-((5-nitrofuran-2-yl)methylene) benzohydrazide **3h.** M.p. 234-236°C; Yield - 88%; ^{1}H NMR (400 MHz, DMSO) δ in ppm: 12.07 (s, 1H, NH), 8.43 (s, 1H, =CH-Ar), 7.80-7.79(d, 1H, Ar-H), 7.27-7.24 (m, 3H, Ar-H), 3.86 (s, 6H, -OCH_3), 3.73 (s, 3H, -OCH_3); IR (cm^{-1}) ν_{max} 3250 (N-H), 2995 (C-H) 1653 (C=O), 1596,1498 (Ar-C=C), 1557 (*asym*, Ar-NO_2), 1340 (*sym*, Ar-NO_2), 1244 (C-O-C); ESI-MS *m/z* : 372 (M+Na$^+$).

2-(naphthalen-1-yl)-N'-((5-nitrofuran-2-yl)methylene) acetohydrazide **3i.** M.p. 206-207°C; Yield - 85%; ^{1}H NMR (400 MHz, DMSO) δ in ppm: 12.08 (s, 1H, NH), 8.23 (s, 1H, =CH-Ar), 8.06-7.75(m, 4H, Ar-H), 7.56-7.43(m, 4H, Ar-H), 7.25-7.19 (m, 1H, Ar-H), 4.43 (s, 2H, -CH_2); IR (cm^{-1}) ν_{max} 3210 (N-H), 2991 (C-H) 1665 (C=O), 1507,1475 (Ar-C=C), 1558 (*asym*, Ar-NO_2), 1351 (*sym*, Ar-NO_2); ESI-MS *m/z* : 346 (M+Na$^+$).

3-bromo-N'-(4-nitrobenzylidene) benzohydrazide **4a.** M.p. 236-238°C; Yield - 85%; ^{1}H NMR (400 MHz, DMSO) δ in ppm: 12.15 (s, 1H, NH), 8.58 (s, 1H, =CH-Ar), 8.21-8.16 (d, 2H, Ar-H), 8.01-7.99 (d, 2H, Ar-H), 7.80-7.51 (m, 4H, Ar-H); IR (cm^{-1}) ν_{max} 3167 (N-H), 3003 (C-H) 1653 (C=O), 1596 (Ar-C=C), 1519 (*asym*, Ar-NO_2), 1339 (*sym*, Ar-NO_2); ESI-MS *m/z* : 370 (M+Na$^+$).

4-bromo-N'-(4-nitrobenzylidene) benzohydrazide **4b**. M.p. 244-246°C; Yield - 87%; ^{1}H NMR (400 MHz, DMSO) δ in ppm: 12.19 (s, 1H, NH), 8.54 (s, 1H, =CH-Ar), 8.31-8.29 (d, 2H, Ar-H), 8.01-7.99 (d, 2H, Ar-H), 7.79-7.75 (m, 4H, Ar-H); IR (cm^{-1}) ν_{max} 3210 (N-H), 2991 (C-H) 1644 (C=O), 1590 (Ar-C=C), 1512 (*asym*, Ar-NO_2), 1349 (*sym*, Ar-NO_2); ESI-MS *m/z* : 370 (M+Na$^+$).

2-bromo-N'-(4-nitrobenzylidene) benzohydrazide **4c.** M.p. 264-266°C; Yield - 83%; ^{1}H NMR (400 MHz, DMSO) δ in ppm: 12.27 (s, 1H, NH), 8.67 (s, 1H, =CH-Ar), 8.30-8.28 (d, 2H, Ar-H), 8.02-7.99 (d, 2H, Ar-H), 7.79-7.75 (m, 4H, Ar-H); IR (cm^{-1}) ν_{max} 3172 (N-H), 3009 (C-H) 1651 (C=O), 1586 (Ar-C=C), 1522 (*asym*, Ar-NO_2), 1339 (*sym*, Ar-NO_2); ESI-MS *m/z* : 370 (M+Na$^+$).

N'-(4-nitrobenzylidene)benzohydrazide **4d.** M.p. 215-217°C; Yield - 85%; ^{1}H NMR (400 MHz, DMSO) δ in ppm: 12.13 (s, 1H, NH), 8.55 (s, 1H, =CH-Ar), 8.31-8.29 (d, 2H, Ar-H), 8.00-7.91 (m, 4H, Ar-H), 7.63-7.52 (m, 3H, Ar-H); IR (cm^{-1}) ν_{max} 2930 (C-H), 1646 (C=O), 1590,1491 (Ar-C=C), 1517 (*asym*, Ar-NO_2), 1341 (*sym*, Ar-NO_2); ESI-MS *m/z* : 292 (M+Na$^+$).

2,4-dichloro-N'-(4-nitrobenzylidene)benzohydrazide **4e.** M.p. 206-207°C; Yield - 89%; ^{1}H NMR (400 MHz, DMSO) δ in ppm: 12.24 (s, 1H, NH), 8.37(s, 1H, =CH-Ar), 8.31-8.17 (m, 2H, Ar-H), 8.00-7.98 (s,1H, Ar-H), 7.78-7.53 (m, 4H, Ar-H); IR (cm^{-1}) ν_{max} 3209 (N-H), 2954 (C-H), 1671 (C=O), 1580,1473 (Ar-C=C), 1521 (*asym*, Ar-NO_2), 1338 (*sym*, Ar-NO_2); ESI-MS *m/z* : 360 (M+Na$^+$).

4-methoxy-N'-(4-nitrobenzylidene)benzohydrazide **4f.** M.P. 260-262°C; Yield - 82%; ^{1}H NMR (400 MHz, DMSO) δ in ppm: 12.00 (s, 1H, NH), 8.53(s, 1H, =CH-Ar), 8.30-8.28 (d, 2H, Ar-H), 7.98-7.91 (m, 4H, Ar-H), 7.09-7.05 (d, 2H, Ar-H), 3.84 (s, 3H, -OCH_3); IR (cm^{-1}) ν_{max} 3236 (N-H), 2963 (C-H), 1649 (C=O), 1603 (Ar-C=C), 1507 (*asym*, Ar-NO_2), 1345 (*sym*, Ar-NO_2), 1256 (C-O-C); ESI-MS *m/z* : 322 (M+Na$^+$).

2-(naphthalen-1-yl)-N'-(4-nitrobenzylidene)acetohydrazide **4g.** M.p. 258-260°C; Yield - 87%; ^{1}H NMR (400 MHz, DMSO) δ in ppm: 12.00 (s, 1H, NH), 8.36 (s, 1H, =CH-Ar), 8.28-8.25(d, 2H, Ar-H), 7.84-7.82(d, 1H, Ar-H), 7.98-7.82 (m, 4H, Ar-H), 7.54-7.45 (m, 4H, Ar-H), 4.50 (s, 2H, -CH_2); IR (cm^{-1}) ν_{max} 3184 (N-H), 2960 (C-H), 1667(C=O), 1602 (Ar-C=C), 1519 (*asym*, Ar-NO_2), 1351 (*sym*, Ar-NO_2); ESI-MS *m/z* : 356 (M+Na$^+$).

3,4,5-trimethoxy-N'-(4-nitrobenzylidene)benzohydrazide **4h.** M.p. 212-214°C; Yield - 84%; ^{1}H NMR (400 MHz, DMSO) δ in ppm: 11.98 (s, 1H, NH), 8.56 (s, 1H, =CH-Ar), 8.32-8.29 (d, 2H, Ar-H), 8.00-7.98 (d, 2H, Ar-H), 7.25 (s, 2H, Ar-H), 3.86 (s, 6H, OCH_3), 3.73 (s, 3H,

OCH_3); IR (cm^{-1}) ν_{max} 2973 (C-H), 1645(C=O), 1581 (Ar-C=C), 1522 (*asym*, Ar-NO_2), 1331 (*sym*, Ar-NO_2), 1232 (C-O-C); ESI-MS *m/z* : 382 ($M+Na^+$).

4-methyl-N'-(4-nitrobenzylidene)benzohydrazide **4i.** M.p. 221-223°C; Yield - 87%; ^{1}H NMR (400 MHz, DMSO) δ in ppm: 12.06 (s, 1H, NH), 8.55 (s, 1H, =CH-Ar), 8.31-8.29 (d, 2H, Ar-H), 7.99-7.97 (d, 2H, Ar-H), 7.85-7.83 (d, 2H, Ar-H), 7.35-7.33 (d, 2H, Ar-H), 3.29 (s, 3H, CH_3); IR (cm^{-1}) ν_{max} 3275 (N-H), 2971 (C-H), 1644(C=O), 1604 (Ar-C=C), 1508 (*asym*, Ar-NO_2), 1347 (*sym*, Ar-NO_2); ESI-MS *m/z* : 306 ($M+Na^+$).

Evaluation of antimycobacterial activity

Determination of MIC: The synthesized compounds after confirmation of their structures were subjected to biological evaluation. The minimum inhibitory concentration (MIC) was determined by dilution method. The procedure followed for anti-TB activity mainly involves the use of Middlebrook 7H11 agar with OADC growth supplement and standard strain of *M.tb* H_{37}Rv (ATCC 27294). The basal medium is prepared and sterilized by autoclaving. The stock solution of the test compounds and standard drugs (Isoniazid, Rifampicin & ethambutol) was prepared in dimethyl sulfoxide (DMSO). Two fold serial dilutions of each test compound/drug were incorporated into Middlebrook 7H11 agar medium with OADC growth supplement. Inoculum of *M. tuberculosis* H_{37}Rv were prepared from fresh Middlebrook 7H11 agar slants with OADC growth supplement adjusted to 1mg/mL in Tween 80 (0.05%) saline diluted to 10^{-2} to give a concentration of approximately 10^7 cfu/mL. The tubes were incubated at 37°C. Along with this one growth control without compound and drug controls were also set up. The tubes are inspected for growth twice a week for a period of three weeks and final readings were recorded after 28 days. The minimum inhibitory concentration (MIC) is defined as the minimum concentration of compound required to inhibit the growth of microorganism.

QSAR Study

In an attempt to determine the role of structural features which appears to influence the observed activity of substituted N'-(4-nitrobenzylidene)/ N'-((5-nitrofuran-2-yl) methylene) benzohydrazide derivatives (*3a-3i &4a-4i*), QSAR studies were undertaken using *SQ-MLR* model. The pMIC value of the biological activity data (excluding two compounds, which MIC value numerically not well defined) was used as dependent variable in QSAR study. These were correlated with different molecular descriptors like constitutional, topological, geometrical, charge, GETAWAY (Geometry, Topology and Atoms-Weighted AssemblY), WHIM (Weighted Holistic Invariant Molecular descriptors), 3D-MoRSE (3D-Molecular Representation of Structure based on Electron diffraction), molecular walk counts, BCUT descriptors, 2D-Autocorrelations, aromaticity indices, Randic molecular profiles, radial distribution functions.

Molecular modeling study was carried out through *CS ChemOffice*,[23] *DRAGON*[24] and *VALSTAT*[25] software. The structure of *N'*-(4-nitrobenzylidene)/ *N'*-((5-nitrofuran-2-yl)methylene) benzohydrazides were subjected to energy minimization using molecular mechanics (MM2) until the RMS gradient value became smaller than 0.419 kJ mol^{-1} $Å^{-1}$. The energy minimized molecules were subjected to re-optimization via AM1 method until the RMS gradient attains a value smaller than 0.419 J mol^{-1} $Å^{-1}$ using MOPAC. The geometry optimization of the lowest energy structure was carried out using EF routine. The QSAR descriptors of substituted hydrazides were calculated using the molecular package *DRAGON*. Further, the regression analysis was performed using the statistical program *VALSTAT*.

The statistical fitness of the regression equations were assessed by parameters like Pearson correlation coefficient (*r*), coefficient of determination (r^2), explained variance (r^2_{adj}), standard error of estimate (*SEE*), and sequential Fischer test (*F*) at specified degree of freedom (*df*). The quality of the regression equations were analyzed by parameters such as quality factor (*QF*), pair-wise correlation (*PWC*), variance inflation factor (*VIF*), probable error of coefficient of determination (*PE*), outlier (Z_{value}), Akaike's information criterion (*AIC*) and Kubinyi function (*FIT*).

The robustness of the regression equation was ascertained through bootstrapping analysis and response scrambling analysis (*Y*-randomization test) techniques. Bootstrapping squared correlation coefficient (r^2_{BS}) is the average squared correlation coefficient of subset of compounds used in regression, while bootstrapping standard deviation ($r^2_{BS\_STD}$) is the standard deviation of n run data of bootstrapping method. Response scrambling analysis was evaluated by parameters like randomized squared correlation coefficient ($r^2_{RAND\_MAX}$), mean randomized squared correlation coefficient ($r^2_{RAND\_MEAN}$), standard deviation of randomized squared correlation coefficient ($r^2_{RAND\_STD}$) and chance correlation factor (*Chance*).

Validation of QSAR models

The internal predictive powers of the equations were validated by leave-n-out (*lno*) cross-validation method[26,27] considering predicted residual sum of square (*PRESS*), total sum of squares (*SSY*), cross-validated squared correlation coefficient (Q^2), standard error of predicted residual sum of square (S_{PRESS}) and standard error of prediction (S_{DEP}).

A model is built with N - 1 compounds and N^{th} compound is predicted. Each compound is left out of the model derivation and predicted in turn an indication of the performance is obtained from cross-validated (or predictive Q^2) method which is defined as

$$Q^2 = 1 - \sum (Y_{predicted} - Y_{actual})^2 / (Y_{actual} - Y_{mean})$$

Where, $Y_{predicted}$, Y_{actual}, and Y_{mean} are the predicted, actual and mean values of target property (pMIC), respectively. R $(Y_{predicted} - Y_{actual})^2$ is the predictive residual error sum of squares.

Calculation of statistical parameters

The selected models were also validated by the calculation of following statistical parameters: [28] Probable error of the coefficient of correlation (*PE*), Friedman's lack of fit measure (*LOF*), standard error of prediction (S_{DEP}), and variance inflation factor.

These parameters were calculated from the following equations.

$$PE = 2 (1 - r^2)/3\sqrt{n}$$

Where, *r* is the correlation coefficient and n is the number of compounds used.

$$LSE = \sum (Y_{obs} - Y_{cal})^2$$

Where, Y_{obs} and Y_{calc} are the observed and calculated values, respectively.

$$LOF = LSE/ \{1 - (C + d.\ p/n)\}^2$$

where LSE is the least square error, C is the number of descriptors + 1, p is the number of independent parameters, n is the number of compounds used, d is the smoothing parameter which controls the bias in the scoring factor between equations with different number of terms and was kept 1.0.

$$S_{DEP} = \sqrt{LSE/n}$$

Variation inflation factor is employed to determine the multicolinearity between the physicochemical parameters. The VIF value is calculated as

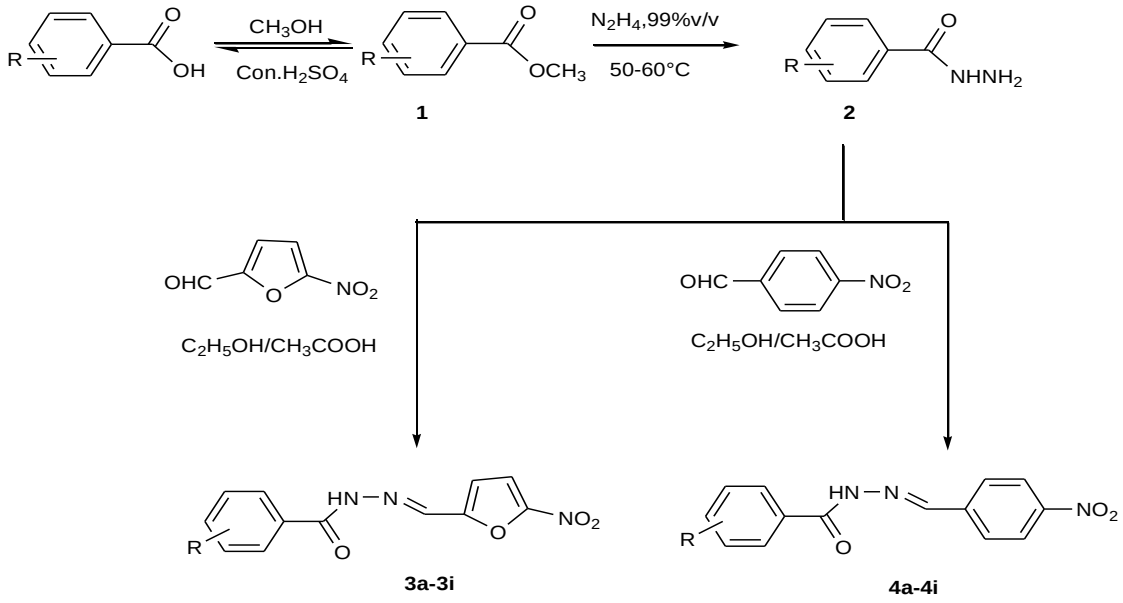
$$VIF = 1 / 1 - r^2$$

Where, r^2 is the squared multiple correlation coefficient of one parameter effect on the remaining parameter. VIF values greater than 5 indicate the presence of unacceptably large multicolinearity between parameters in the correlation.

RESULTS AND DISCUSSION

The synthesis of intermediate and target compounds was performed according to reactions outlined in **Scheme 1**. Esterification of various substituted benzoic acid with methyl alcohol was carried out in the presence of sulfuric acid yielded corresponding benzoic acid

esters, subsequently reaction of ester with hydrazine hydrate afforded the corresponding hydrazides. Substituted hydrazides were condensed with different substituted aldehydes to yield the substituted benzoic acid benzylidene/ 5-nitrofuran-2-yl-methylene hydrazides (**3a-3i & 4a-4i**). The progress of reactions monitored with the help of thin layer chromatography. The products so obtained were purified by flash column chromatography technique. The target compounds were obtained in appreciable yield and their physicochemical characteristics are presented in Table 1.



Scheme 1: Synthesis of substituted *N*′-(4-nitrobenzylidene) / *N*′-((5-nitrofuran-2-yl)methylene)benzohydrazide analogs (3a-3i & 4a-4i)

Table 1: Physicochemical characteristics of synthesized substituted *N*′-(4-nitrobenzylidene) / *N*′-((5-nitrofuran-2-yl)methylene) benzohydrazide analogs.

Comp. no	Mol.Formula	Mol.Weight	M.P (°c)	[a]R_f	λ_{max}	%Yield
3a	$C_{12}H_9N_3O_4$	259.22	203-204	0.72	352	80
3b	$C_{12}H_8BrN_3O_4$	338.11	206-208	0.75	354	82
3c	$C_{12}H_8BrN_3O_4$	338.11	200-201	0.77	359	84
3d	$C_{12}H_8BrN_3O_4$	338.11	224-226	0.71	358	85
3e	$C_{12}H_7Cl_2N_3O_4$	328.11	210-211	0.78	353	87
3f	$C_{13}H_{11}N_3O_5$	289.24	>270	0.73	364	86
3g	$C_{13}H_{11}N_3O_4$	273.24	238-240	0.69	360	81
3h	$C_{15}H_{15}N_3O_7$	349.3	234-236	0.79	362	88
3i	$C_{17}H_{13}N_3O4$	323.3	206-207	0.75	357	85
4a	$C_{14}H_{10}BrN_3O_3$	348.15	236-238	0.65	327	85
4b	$C_{14}H_{10}BrN_3O_3$	348.15	244-246	0.68	328	87
4c	$C_{14}H_{10}BrN_3O_3$	348.15	264-266	0.65	320	83
4d	$C_{14}H_{11}N_3O_3$	269.26	215-217	0.59	328	85
4e	$C_{14}H_9Cl_2N_3O_3$	338.15	206-207	0.61	319	89
4f	$C_{15}H_{13}N_3O_4$	299.28	260-262	0.64	334	82
4g	$C_{19}H_{15}N_3O_3$	333.34	258-260	0.58	323	87
4h	$C_{17}H_{17}N_3O_6$	359.33	212-214	0.63	333	84
4i	$C_{15}H_{13}N_3O_3$	283.28	221-223	0.70	325	87

[a] Mobile phase – Hexane : Ethyl acetate

The structures of compounds (**3a-3i & 4a-4i**) were assigned by IR and ^{1}H-NMR spectroscopic data, which are consistent with the proposed molecular structures. The presence of C=O stretching of amide group was confirmed by appearance of bands at 1644-1678 cm^{-1} in IR spectra. The appearance of stretching band around 3200 cm-1 indicates the presence of NH linkage in the synthesized hydrazide derivatives. The presence of peaks slightly above and below 3000 cm^{-1} indicates the presence of aromatic portion in synthesized compounds, respectively. The aromatic nitro stretching bands at 1319-1350 cm^{-1} (symmetric NO_2 stretching) and 1507-1557 cm^{-1} (asymmetric NO_2 stretching) depicts the presence of nitro functional group.

^{1}H-NMR spectral study gave the multiplet signal between 7.05-8.32δ ppm, which is indicative of aromatic proton. The compound showed singlet at δ 2.38 ppm due to the CH_3 group (Ar-CH_3), and a singlet at δ 3.73-3.86 ppm was observed for methoxy (Ar-OCH_3) group protons. The presence of singlet at δ 11.98-12.31ppm indicated the presence of -NH protons of compound. Further the presence of the N=CH fragment was indicated by appearance of a signal at δ 8.23-8.67ppm. Result of spectral analysis confirmed the formation of anticipated hydrazide analogs.

Antimycobacterial activity

The *in vitro* antimycobacterial activity of synthesized compounds against MTB was carried out in Middlebrook 7H11agar medium supplemented with OADC by agar dilution method and the results are presented in Table 2 & Figure 1. At the commencement of this study in the preliminary screening, 5-nitrofuran-2-yl-methylene substituted derivatives displayed relatively higher inhibitory activity as compared to 4-nitrobenzylidene substituted derivatives. The activity of 5-nitrofuran-2-yl methylene analogs ranges from 0.781 to 12.5 µg/mL while 4-nitrobenzylidene analogs varies from 6.25 to more than 50µg/mL. 2,4-dichloro-*N*′-((5-nitrofuran-2-yl)methylene) benzohydrazide (**3e)** is found to be the most active against *M. Tuberculosis* with MIC value 0.78 µg/mL. Whereas compound **3d, 3f**

and **3i** shown MIC value 1.56, 3.13 and 3.13 µg/mL respectively. *m*-bromo substituted analogs of 5-nitrofuran-2-yl-methylene/4-nitrobenzylidene benzohydrazide are less potent as compare to other derivatives while *p*-bromo substituted analogs of *N'*-((5-nitrofuran-2-yl)methylene)benzohydrazide was equipotent to ethambutol. 2-(naphthalen-1-yl)-*N'*-((5-nitrofuran-2-yl)methylene) acetohydrazide (**3i**) are more potent with MIC value 3.13 µg/mL in comparison with their N'-(4-nitrobenzylidene) analogs (**4g**; MIC: 12.5 µg/mL). The results are compared with that of standard isoniazid, rifampicin and ethambutol.

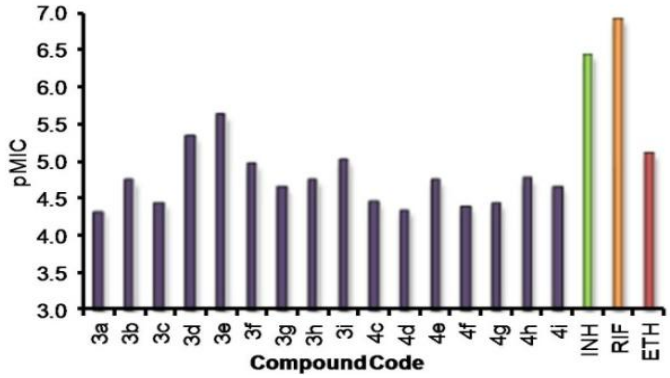
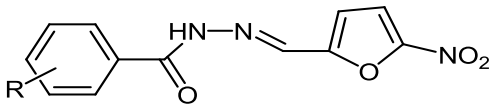
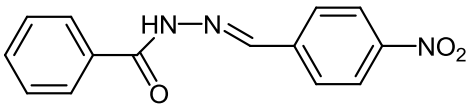


Figure 1: Graphical representation of negative logarithmic molar concentration of MIC of the *N'*-((5-nitrofuran-2-yl/4-nitrophenyl) methylene) substituted hydrazides against *M. tuberculosis*.

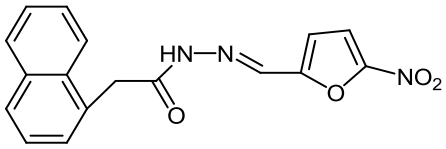
Table 2: Structure and biological activity of substituted *N'*-(4-nitrobenzylidene)- benzohydrazide / *N'*-((5-nitrofuran-2-yl)methylene) benzohydrazide analogs



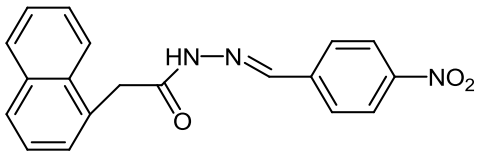
3a-3h



4a-4i



3i



4g

Comp. no	R	[a]*MIC (µg/mL)*	[b]*MIC (µM)*	[c]*pMIC*
3a	-H	12.5	48.218	4.317
3b	2-Br	6.25	18.484	4.733
3c	3-Br	12.5	36.968	4.432
3d	4-Br	1.56	4.622	5.335
3e	2,4-Cl	0.78	2.380	5.623
3f	4-OCH_3	3.13	10.803	4.966
3g	4-CH_3	6.25	22.871	4.641
3h	3,4,5-OCH_3	6.25	17.891	4.747
3i	-	3.13	9.665	5.015
4a	3-Br	>50	-	-
4b	4-Br	>50	-	-
4c	2-Br	12.5	35.902	4.445
4d	-H	12.5	46.420	4.333
4e	2,4-Cl	6.25	18.482	4.733
4f	4-OCH_3	12.5	41.763	4.379
4g	-	12.5	37.496	4.426
4h	3,4,5-OCH_3	6.25	17.392	4.760
4i	4-CH_3	6.25	22.061	4.656
Isoniazid	-	0.049	0.36	-
Rifampicin	-	0.098	0.12	-
Etambutol	-	1.56	7.64	-

[a]Minimum inhibitory concentration against *M. tuberculosis* in µg/mL; [b]Minimum inhibitory concentration in µM; [c] Negative logarithmic minimum inhibitory concentration in mole

Preliminary structure activity relationship analysis revealed that 5-nitrofuran-2-yl-methylene analogs more potent than 4-nitrobenzylidene substituted analogs. Meta substitution on benzohydrazide moiety is unfavorable towards reactivity. Electron withdrawing group at *ortho* and *para* position is favorable for the anti-mycobacterial activity in 5-nitrofuran-2-yl-methylene analogs. The obtained result revealed that the nature of substituents have a considerable impact on antimycobacterial activity of target hydrazones and following structure activity relationship (SAR) can be deduced:

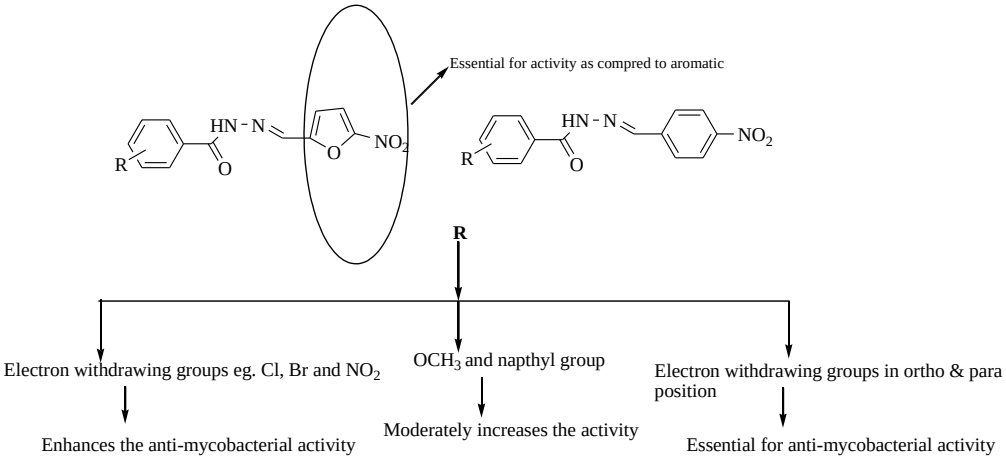


Figure 2: Structural requirements for antimycobacterial activity of substituted hydrazide derivatives

Structure Activity Relationship (SAR)

1. The analysis of antimycobacterial results indicated that the compounds of 5-nitrofuran-2-yl-methylene substituted derivatives having relatively higher inhibitory activity as compared to 4-nitrobenzylidene substituted derivatives. It is concluded that the 5-nitrofuran-2-yl moiety is essential for the antimycobacterial activity. This fact is supported by the observations of Tangallapally *et al*.[29]
2. The synthesized compound (**3e**) derived from dichlorobenzoic acid and 5-nitrofurfuraldehyde displayed greater activity as compared to 4-nitrobenzylidene derivative. The presence of electron-withdrawing groups in 5-nitrofuran-2-yl moiety enhancing the antimycobacterial activity.
3. The presence of electron-withdrawing bromo (compounds **3b, c, & d**) and chloro (**3e**) groups in 5-nitrofuran-2-yl-methylene enhance the growth inhibition potency as compared to 4-nitrobenzylidene analogs. Electron withdrawing group at *ortho* and *para* position is favorable for the antimycobacterial activity in 5-nitrofuran-2-yl-methylene analogs.
4. The presence of methoxy group and napthyl ring (compounds **3f, h & i**) were endowed with moderate antimycobacterial activity. The importance of electron-donating groups in 5-nitrofuran-2-yl- methylene analogs moderately enhancing the activity as compared to 4-nitrobenzylidene analogs.
5. The presence of an electron-withdrawing NO_2 group in the furanyl analogs make them highly potent antimycobacterial agents. The role of an electron-withdrawing group in increasing the antimicrobial potency is similar to the results of Sharma *et al*.[30]
6. It is interesting to note that the compound **3d** is equipotent to ethambutol (MIC of 1.56 µg/mL). The replacement of NH_2 group of hydrazide with heteroaromatic and aromatic ring (**3a-i &4a-i**) led to a perceptible increase in antimycobacterial activity of the synthesized compounds.
7. From the abovementioned discussion concluded that different structural requirements are essential for a compound to be selected as antimycobacterial agents. The SAR results are summarized in Fig. 2.

QSAR analysis of hydrazide analogs

In order to identify substituent effect on antimycobacterial activity, quantitative structure-activity relationship (QSAR) studies of title compounds were performed. The data set of 16 (excluding two compounds, which MIC value numerically not well defined) compounds were subjected to QSAR analysis using multiple linear regression (MLR) analysis in order to explore responsible molecular descriptors for antimycobacterial activity. Biological activity data (MIC) were used after adapting negative logarithm in molar concentration. Out of hundreds of equations generated, some of the best QSAR equations having significant statistical qualities are selected and the descriptors used in these equations selected for the QSAR study. These equations were generated by sequential multiple linear regression (*SQMLR*) approach. In *SQMLR*, the program searches all the permutation/combination for the given data set sequentially.

The selected QSAR model is shown as Eqn. 1.

$$pMIC = 0.170(\pm0.020)RDF135m + 0.359(\pm0.043)RDF030v - 0.068(\pm0.016)RDF120u + 3.783$$

$$n = 16,\ r = 0.946,\ r^2_{adj} = 0.868,\ SEE = 0.133,\ F = 33.962 \quad \text{.....(1)}$$

Quality parameters:

QF = 7.087, *PE* = 0.018, *FIT* = 4.075, *LOF* = 0.675, *AIC* = 0.030, *PWC* < 0.350, *VIF* <1.250, *Outlier* = Nil

Validation parameters

r^2_{BS} = 0.908, $r^2_{BS\_STD}$ = 0.092, *Chance* < 0.001, $r^2_{RAND\_MAX}$ = 0.835, $r^2_{RAND\_MEAN}$ = 0.192, $r^2_{RAND\_STD}$ = 0.143, ${}_1Q^2$ = 0.760, ${}_1S_{PRESS}$ = 0.201, ${}_1S_{DEP}$ = 0.174, ${}_5Q^2$ = 0.817, ${}_5S_{PRESS}$ = 0.156, ${}_5S_{DEP}$ = 0.152

QSAR model (Eq. 1) shows correlation coefficient (*r*) of 0.946, which accounts for 86.8% of the explain variance in the activity. QSAR model shows significance level more than 99.9% as it exceeded the tabulated $F_{(3,12\ \alpha\ 0.001)}$ = 12.7.

Preliminary analysis was carried out in terms of pair wise correlation analysis (*PWC*). A correlation analysis was performed on all the descriptors, depending on the inter-correlation among the independent descriptors and also their individual correlation with antimycobacterial activity. The correlation matrix elicited in Table 3. From the correlation matrix it was observed that there is low value of pair wise correlation (≤ 0.5) between the descriptors suggested that independent contribution.

Table 3: Pair wise correlation and *VIF* values of the descriptors used in QSAR models

Eq.	*Descriptors*	*VIF*	*Pair wise correlation (PWC)*		
			RDF120u	***RDF135m***	***RDF030v***
	RDF120u	1.116	1.000		
1	***RDF135m***	1.248	0.244	1.000	
	RDF030v	1.189	0.113	0.343	1.000

The orthogonality of the descriptors in the model was established through variance inflation factor (*VIF*)[31,32] values (Table 3). *VIF* value larger than 10 indicates that the information of the descriptors may be hidden by the correlation of the other descriptors. *VIF* is less than 1.3 for all the contributing descriptors revealed that the descriptors are fairly independent to each other.

In order to confirm the results, the comparison of observed and calculated values demonstrated that they are close to each other (Table 4 & Fig 3). In present study efforts was done to investigate

predictive power of the proposed model by using quality factor (*QF*)[33,34]. The larger value of *QF* (7.087) signifies better predictive power of the model. Goodness of fit is calculated as *PE*,[35] the selected model, have correlation coefficient value significantly higher than 6*PE* supporting reliability and goodness.

Table 4: Observed, calculated, calculate (leave one out), residual and Z-score value of hydrazone analogs

Comp. No.	pMIC					
	[a]Obs	[b]Cal	[c]Res_{cal}	Z_{score}	[d]$Cal_{(LOO)}$	[e]$Res_{Cal(LOO)}$
3a	4.317	4.250	0.067	0.557	4.235	0.082
3b	4.733	4.743	-0.010	-0.086	4.745	-0.012
3c	4.432	4.680	-0.248	-2.079	4.736	-0.304
3d	5.335	5.093	0.242	2.025	5.042	0.293
3e	5.623	5.705	-0.081	-0.680	5.888	-0.265
3f	4.966	4.962	0.004	0.036	4.961	0.005
3g	4.641	4.698	-0.058	-0.482	4.706	-0.065
3h	4.747	4.791	-0.043	-0.363	4.802	-0.054
3i	5.015	4.979	0.036	0.300	4.966	0.049
4c	4.445	4.567	-0.122	-1.021	4.585	-0.140
4d	4.333	4.369	-0.036	-0.302	4.383	-0.049
4e	4.733	4.528	0.205	1.716	4.490	0.243
4f	4.379	4.475	-0.096	-0.804	4.509	-0.130
4g	4.426	4.412	0.014	0.117	4.410	0.016
4h	4.760	4.680	0.080	0.667	4.412	0.347
4i	4.656	4.609	0.048	0.400	4.601	0.055

[a] Observed data of the compounds used in generation of model; [b] Calculated data of the compounds using model; [c] Residual value of calculated data; [d] Calculate (LOO) data of the compounds using leave-one-out method; [e] Residual value of calculated (LOO) data.

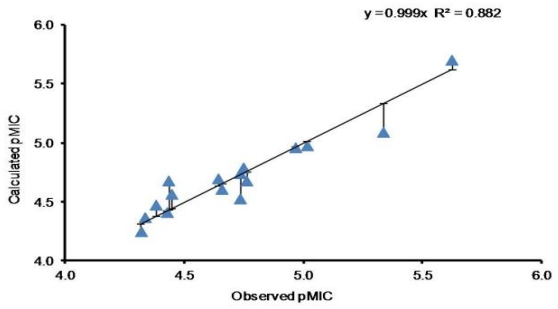


Figure 3: A graphical representation of observed and calculated p*MIC* of hydrazides

Additional statistical parameters, such as the Akaike's information criterion (*AIC*),[36,37] the Kubinyi function (*FIT*)[38,39] and the Friedman's lack of fit (*LOF*),[40] have been calculated to further validate the derived models. The *AIC* takes into account the statistical goodness of fit and the number of parameters that have to be estimated to achieve that degree of fit. The *FIT*, closely related to the *F*-value, proved to be a useful parameter for evaluating the quality of the models. A model which is derived in k independent descriptors, its *F*-value will be more sensitive if k is small while it becomes less sensitive if k is large. The *FIT*, on the other hand, will be less sensitive if k is small whereas it becomes more sensitive if k is large. The model that produces the lowest *AIC* value and highest *FIT* value is considered potentially the most useful and the best. The *LOF* factor takes into account the number of terms used in the equation and is not biased, as are other indicators, toward large number of parameters. The lower values of parameters *AIC* (0.030) and *LOF* (0.675) and higher value of *FIT* (4.075) supported ascendancy of this model over other regression expressions. The equation was further tested for outlier by the Z-score method and no compound was found to be an outlier, suggesting that the equation is able to explain the structurally diverse analogues of the series.

Preliminarily selected regression expressions have been further internal validated using Response scrambling analysis test[41] by repeated randomization of the activity to discover the chance correlations, if any, associated with them. The scrambled activity analysis revealed that chance correlation factor (*Chance* < 0.001) having value less than 0.001 i.e. the result was not based on prospective correlation. Similarly max randomized r^2 ($r^2_{RAND\_MAX}$), mean randomized r^2 ($r^2_{RAND\_MEAN}$) and randomized standard deviation ($r^2_{RAND\_STD}$) values are also supportive of the fact that the results are not based on chance correlation.

Bootstrapping analysis was performed for further access of the robustness and statistical confidence. In bootstrapping repetitively analyzed sub-samples of the data. Each sub-sample is a random sample with replacement from the full sample. One data point can be represented more than once or not at all but the total number of data points should remain constant. The bootstrapping analysis gives an overview about contribution of individual molecules to the QSAR model. In present study 1000 sub-samples were used for internal validation. The r^2_{BS} is the average squared correlation coefficient calculated during the validation procedure which is computed from a subset of compounds used one at a time for the validation procedure. $r^2_{BS\_STD}$ is the standard deviation in multiple run of a given data set. If the value of r^2_{BS} is at par to conventional r^2 and $r^2_{BS\_STD}$ is low than the model is robust and promising. In our cram both values of each expression fall within the limit.

The internal reliability of the model was confirmed from leave-one-out (LOO)[26] and leave-five-out procedures using cross-validated squared correlation coefficient (Q^2), standard error of prediction (S_{PRESS}) and standard deviation of prediction (S_{DEP}) respectively. In leave-five-out procedure a group of five compounds are kept outside the analysis each time in such a way that all compounds, for once, become the part of the predictive groups. The reasonable QSAR model, should have Q^2 value greater than 0.5, and a value of this index for ${}_1Q^2 = 0.760$ and ${}_5Q^2 = 0.817$ indicates good predictivity.

In order to confirm the results, the comparison of observed and calculated (LOO) values demonstrated that they are close to each other evidenced by the low residual activity data (Table 4 & Fig 4). The statistical data of the model depicted that it's fulfill the validation criteria to a significant degree.

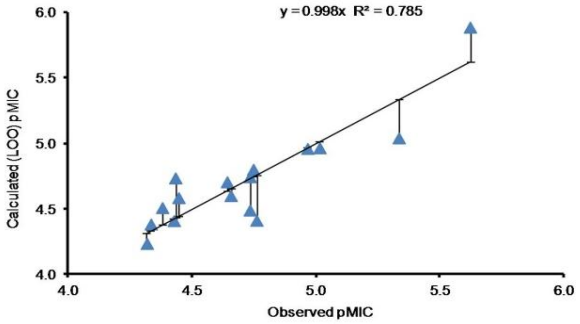


Figure 4: A graphical representation of observed and Calculated (leave one out) p*MIC* of hydrazides

The QSAR model obtained by linear regression analysis divulged that radial distribution functions are able to explain the antimycobacterial activity of hydrazide analogs. The statistically significant model indicates that RDF0135*m* and RDF030*v* are contributed positively while RDF120*u* contributed negatively. RDF descriptors[42-44] is belonging to the class of radial distribution function, which is based on the distance distribution in the geometrical representation of the molecule. In addition to inter-atomic distances in the entire molecule, the RDF also provides valuable information about bond distances, ring types, planar and non-planar systems, atom types and other important structural motifs.

The RDF code has been proven to be a good representation for the 3D structure which has several merits like independence from the number of atoms; unambiguity regarding the three-dimensional arrangement of the atoms and invariance against translation and rotation of the entire molecule. The RDF of an ensemble of N atoms can be interpreted as the probability distribution to find an atom in a spherical volume of radius R. The general form of the radial distribution function code (RDF code) is represented by:

$$g(R) = f\sum_{i=1}^{N-1}\sum_{j=i+1}^{N}\omega_i\omega_j e^{-\beta(R-r_{ij})^2}$$

Where *f* is a scaling factor, *N* is the number of atoms, ω is the atomic properties of atoms *i* and *j*, β is the smoothing parameter which defines the probability distribution of the individual inter-atomic distance. β can be interpreted as the temperature factor that defines the movement of the atoms. r_{ij} is the distance between the atoms *i* and *j, g(R)* was calculated at a number of discrete points with defined intervals.

A typical RDF descriptor is denoted by RDFsω where 1.0 ≤ s ≤ 15.5 in units of 0.5 and the weights ω ε {u,m,*v*,e,p} as unweighted (u) , weighted by atomic masses (m), weighted by atomic van der Waals volumes (*v*), weighted by atomic Sanderson electronegativities (e) and weighted by atomic polarizabilities (p). *RDF135m* is the radial distribution function at 13.5Å inter-atomic distance weighted by atomic mass and contributed positively, revealed that increase in atomic masses are favorable for the activity. The atomic masses might be helpful for the activity through enthalpy gain by the non-bonded interactions with macromolecule. *RDF030v* is the radial distribution function at 3.0Å inter-atomic distance weighted by atomic van der Waals volumes contributed positively. The contribution of *RDF030v* revealed that van der Waals volume suggested entholpic contribution to the activity and it might be responsible for the interaction with hydrophobic pocket of the macromolecule. *RDF120u* is the radial distribution function at 12.0Å inter-atomic distance unweighted and contributed negatively, depicted that non-specific RDF contribution towards enthalpic or entropic penalties.

CONCLUSIONS

In conclusion, a series of substituted hydrazide derivatives have been synthesized and their *in-vitro* activity was evaluated against *Mycobacterium tuberculosis*. The results of anti-mycobacterial activity study indicated that the presence of halogen substitution at benzohydrazide moiety improved the activity of these derivatives. To understand the relationship between molecular descriptors and anti-mycobacterial activity of substituted hydrazide derivatives, QSAR investigation was performed. The QSAR analysis results indicated the radial distribution function (*RDF030v, RDF135m & RDF120u*) might be helpful in describing the anti-mycobacterial activity of hydrazides. These results make newer hydrazide derivatives as interesting lead molecules for further synthetic and biological evaluation. It can be concluded that this class of compounds certainly holds great promise towards the pursuit to discover new classes of anti-mycobacterial agents.

ACKNOWLEDGMENTS

The authors are grateful to the Director of Shri G. S. Institute of Technology and Science, Indore for providing facilities for this work.

The author R.A.G. is grateful to UGC, New Delhi, for providing senior research fellowship.

REFERENCES

1. WHO Report, Global Tuberculosis Control - Surveillance, planning, financing, 2008.
2. Lourenco, M.C.S.; Ferreira, M.L.; Souza, M.V.N.; Peralta, M. A.; Vasconcelos, T.R.A.; Henriques, M.G.M.O. Synthesis and anti-mycobacterial activity of (E)-N'-(monosubstituted-benzylidene) isonicotinohydrazide derivatives. Eur. J. Med. Chem. 2008, 43, 1344-1347.
3. Bedia, K.K.; Elcin, O.; Seda, U.; Fatma, K.; Nathaly, S.; Sevima, R.; Dimoglo, A. Synthesis and characterization of novel hydrazide-hydrazones and the study of their structure-antituberculosis activity. Eur. J. Med. Chem. 2006, 41, 1253-1261.
4. Kaye, K.; Frieden, T.R. Tuberculosis Control: The relevance of classic principles in an era of acquired immunodeficiency syndrome and multidrug resistance. Epidemiol. Rev. 1996, 18, 52-63.
5. Centers for Disease Control and Prevention. Emergence of Mycobacterium tuberculosis with extensive resistance to second line drugs - worldwide, 2000–2004. MMWR Morb. Mortal. Wkly. Rep. 2006, 55, 301-305.
6. Rollas, S.; Kucukguzel. S.G. Biological activities of hydrazone derivatives. Molecules. 2007, 12, 1910-1939.
7. Bayrak, H.; Demirbas, A.; Demirbas, N.; Karaoglu, S.A. Synthesis of some new 1,2,4-triazoles starting from isonicotinic acid hydrazide and evaluation of their antimicrobial activities. Eur. J. Med. Chem. 2009, 44, 4362-4366.
8. Nayyar, A.; Monga, V.; Malde, A.; Coutinho, E.; Jain, R. Synthesis, anti-tuberculosis activity and 3D-QSAR study of 4-(adamantan-1-yl)-2-substituted quinolines. Bioorg. Med. Chem. 2007, 15, 626-640.
9. Imramovsky, A.; Polanac, S.; Vinsova, J.; Kocevar, M.; Jampitek, J.; Reckova, Z.; Kaustova, J.A. A new modification of anti-tubercular active molecules. Bioorg. Med. Chem. 2007, 15, 2551-2559.
10. Janin, Y. Antituberculosis drugs: Ten years of research. Bioorg. Med. Chem. 2007, 15, 2479-2513.
11. Savini, L.; Chiasserini, L.; Travagli, V.; Pellerano, C.; Novellino, E.; Consentino, S.; Pisano, M.B. New α-(N)-heterocyclichydrazones: evaluation of anticancer, anti-HIV and antimicrobial activity. Eur. J. Med. Chem. 2004, 39, 113-122.
12. Bijev, A. New heterocyclic hydrazones in the search for antitubercular agents: synthesis and in-vitro evaluations. Lett. Drug. Des. Discov. 2006, 3, 506-512.
13. Salgin-Goksen, U.; Gokhan-Kelekci, N.; Goktas, O.; Koysal, Y.; Kılıc, E.; Isik, S.; Aktay, G.; Ozalp, M.L. 1-Acylthiosemicarbazides, 1,2,4-triazole-5(4H)-thiones, 1,3,4-thiadiazoles and hydrazones containing 5-methyl-2-benzoxazolinones: synthesis, analgesic-anti-inflammatory and antimicrobial activities. Bioorg. Med. Chem. 2007, 15, 5738-5751.
14. Kalsi, R.; Shrimali, M.; Bhalla, T.N.; Barthwal, J.P. Synthesis and anti-inflammatory activity of indolyl azetidinones. Ind. J. Pharm. Sci. 1990, 52, 129-134.
15. EI-Sabbagh, O.I.; Rady, H.M. Synthesis of new acridines and hydrazones derived from cyclic β-diketone for cytotoxic and antiviral evaluation. Eur. J. Med. Chem. 2009, 44, 3680-3686.
16. Gemma, S.; Kukreja, G.; Fattorusso, C.; Persico, M.; Romano, M.P.; Altarelli, M.; Savini, L.; Campiani, G.; Fattorusso, E.; Basilico, N.; Taramelli, D.; Yardley, V.; Butini, S. Synthesis of N1- arylidene-N2-quinolyl and N2-acrydinylhydrazones as potent antimalarial agents active against CQ-resistant P. falciparum strains. Bioorg. Med. Chem. Lett. 2006, 16, 5384-5388.
17. Joshi, S.D.; Vagdevi, H.M.; Vaidya, V.P.; Gadaginamath, G.S. Synthesis of new 4-pyrrol-1-yl benzoic acid hydrazide analogs and some derived oxadiazole, triazole and pyrrole ring systems: a novel class of potential antibacterial and

antitubercular agents. Eur. J. Med. Chem. 2008, 43, 1989-1996.
18. Sriram, D.; Yogeeswari, P.; Madhu, K. Synthesis and in vitro antitubercular activity of some 1-[(4-sub)phenyl)-3-(4-{1-((pyridine- 4-carbonyl) hydrazono) ethyl} phenyl) thiourea. Bioorg. Med. Chem. Lett. 2006, 16, 876-878.
19. Yao, X.J.; Panaye, J.P.; Doucet, J.P.; Zhang, R.S.; Chen, H.F.; Lice, M.C.; Hu, Z.D.; Fan, B.T. Comparative study of QSAR/QSPR correlations using support vector machines, radial basis function neural networks, and multiple linear regression. J. Chem. Inf. Comput. Sci. 2004, 44, 1257-1266.
20. Hanch, C.; Leo, A. Exploring QSAR, fundamentals and applications in chemistry and biology. American Chemical Society: Washington, DC, 1995; pp 90-100.
21. Painea, S.W.; Barton, P.; Bird, J.; Denton, R.; Menochet, K.; Smith, A.; Tomkinson, N.P.; Chohan, K.K. A rapid computational filter for predicting the rate of human renal clearance. J. Mol. Graph. Model. 2010, 29, 529-537.
22. Zhoua, Y.P.; Cai, C.B.; Huanb, S.; Jiang, J.H.; Wu, H.L.; Shen, G.L.; Yu, R.Q. QSAR study of angiotensin II antagonists using robust boosting partial least square regression. Anal. Chim. Acta. 2007, 593, 68-74.
23. C.S. ChemOffice, Cambridge Soft Corporation, Software Publishers Association. USA: 1730 M Street, Suite 700, Washington D.C. 20036 (202) 452-1600.
24. Todeschini, R.; Consonni, V. DRAGON-Software for the Calculation of Molecular Descriptors. rel. 1.12 for Windows 2001.
25. Gupta, A.K.; Babu, M.A.; Kaskhedikar, S.G. VALSTAT: Validation program for quantitative structure activity relationship studies. Indian J. Pharm. Sci. 2004, 66, 396-402.
26. Schaper, K.J. Free-Wilson-type analysis of non-additive substituent effects on THPB dopamine receptor affinity using artificial neural networks. Quant. Struct. Act. Relat. 1999, 18, 354-360.
27. Wold, S.; Eriksson, L. In Chemometric Methods in Molecular Design; Water, H.V., Ed.; VCH: Weinheim, 1995; pp 309-318.
28. Pinheiro, A.A.C.; Borges, R.S.; Santos, S.L.; Alves, C.N. A QSAR study of 8.O.4'-neolignans with antifungal activity. J. Mol. Struct. (Theochem). 2004, 672, 215-219.
29. Tangallapally, R.P.; Lee, R.E.B.; Lenaerts, A.J.M.; Lee, R.E. Synthesis of new and potent analogues of anti-tuberculosis agent 5-nitro-furan-2-carboxylic acid 4-(4-benzyl-piperazin-1-yl)-benzylamide with improved bioavailability. Bioorg. Med. Chem. Lett. 2006, 16, 2584-2589.
30. Sharma, P.; Rane, N.; Gurram, V.K. Synthesis and QSAR studies of pyrimido[4,5-d]pyrimidine-2,5-dione derivatives as potential antimicrobial agents. Bioorg. Med. Chem. Lett. 2004, 14, 4185-4190.
31. Chatterjee, S.; Hadi, A.; Price, B. Regression Analysis by Examples, 3rd ed.; Wiley-VCH: New York, 2000.
32. Shapiro, S.; Guggenheim, B. Inhibition of oral bacteria by phenolic compounda. Part. 1. QSAR analysis using molecular connectivity. Quant. Struct. Act. Relat. 1998, 17, 327-337.
33. Pogliani, L. Structure property relationships of amino acids and some dipeptides. Amino Acids. 1994, 6, 141-153.
34. Pogliani, L. Modeling with special descriptors derived from a medium-sized set of connectivity indices. J. Phys. Chem. 1996, 100, 18065-18077.
35. Mandloi, D.; Joshi, S.; Khadikar, P.V.; Khosla, N. QSAR study on the antibacterial activity of some sulfa drugs: building blockers of Mannich bases. Bioorg. Med. Chem. Lett. 2005, 15, 405-411.
36. Akaike, H. Information Theory and an Extension of the Minimum Likelihood Principle. Budapest: Akademiai Kiado, 1973; pp 267-281.
37. Akaike, H. A new look at the statistical model identification. IEEE T. Automat. Contr. 1974, 19, 716-723.
38. Kubinyi, H. Variable selection in QSAR studies. I. An evolutionary algorithm. Quant. Struct. Act. Relat. 1994, 13, 285-294.
39. Kubinyi, H. Variable selection in QSAR Studies. II. A highly efficient combination of systematic search and evolution. Quant. Struct. Act. Relat. 1994, 13, 393-401.
40. Friedman, J. In: Technical Report No. 102. Laboratory for Computational Statistics. Stanford University, Stanford; 1990.
41. So, S.S.; Karplus, M. Three-dimensional quantitative structure-activity relationships from molecular similarity matrices and genetic neural networks. 1. Method and Validations. J. Med. Chem. 1997, 40, 4347-4359.
42. Hemmer, M.C.; Steinhauer, V.; Gasteiger J. The prediction of the 3D structure of organic molecules from their infra spectra. Vib. Spectrosc. 1999, 19, 151-164.
43. Fedorowicz, A.; Zheng, L.; Singh, H.; Demchuk, E. QSAR study of skin sensitization using local lymph node assay data. Int. J. Mol. Sci. 2004, 5, 56-66.
44. Hemmer, M.C.; Gasteiger, J. Prediction of three-dimensional molecular structures using information from infrared spectra. Anal. Chim. Acta. 2000, 420, 145-154.