Arabian Journal of Chemistry (2017) **10**, 41–46

King Saud University

Arabian Journal of Chemistry

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ORIGINAL ARTICLE

Design and synthesis of 5-methylpyrazine-2-carbohydrazide derivatives: A new anti-tubercular scaffold

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Received 14 September 2012; accepted 5 April 2013
Available online 12 April 2013

KEYWORDS

Anti-tubercular;
2D QSAR;
5-Methylpyrazine-2-carbohydrazide;
Mycobacterium tuberculosis

Abstract A simple synthetic methodology was employed for synthesis of series of 5-methylpyrazine-2-carbohydrazide derivatives (PM series). *In vitro* anti-tubercular activity was evaluated against *Mycobacterium tuberculosis* ($H_{37}Rv$) in Middle brook 7H-9 broth medium. Amongst synthesized compounds, seven compounds showed remarkable anti-tubercular activity. The 2-D QSAR illustrates the design PM series of compounds as potential anti-tubercular scaffolds that can be further optimized to improve the activity.

1. Introduction

Tuberculosis is one of the world's great public health threats due to resistance to existing drugs and simultaneous presence of HIV infections. Thus, tuberculosis keeps challenging medicinal chemists to develop new compounds. There are two main strategies for the development of new agents against tuberculosis. The first one requires extraordinary molecular diversity, and the second one is using clinically active and their chemical modification. This second approach is easy and accessible through newly developed computational techniques. The latter strategy rekindled our interest toward pyrazinamide (PZA), which is one of the frontline agents prescribed for the treatment of multidrug resistant tuberculosis (MTB). Recently a proposed mechanism of action has been reported to be the inhibition of the eukaryotic-like fatty acid synthetase I (FASI) of MTB. PZA is considered to be a prodrug of pyrazinoic acid (POA), which is believed to be the active inhibitor of MTB. Activation of PZA to POA was regulated by an enzyme pyrazinamidase present in all PZA-sensitive strains of MTB (World Health Organization Geneva, Switzerland, 2000; Cynamon et al., 1992, 1991; Trnka et al., 1964).

There are two main reasons for selection of pyrazine-2-carbohydrazide as a lead scaffold in this study that are simplicity

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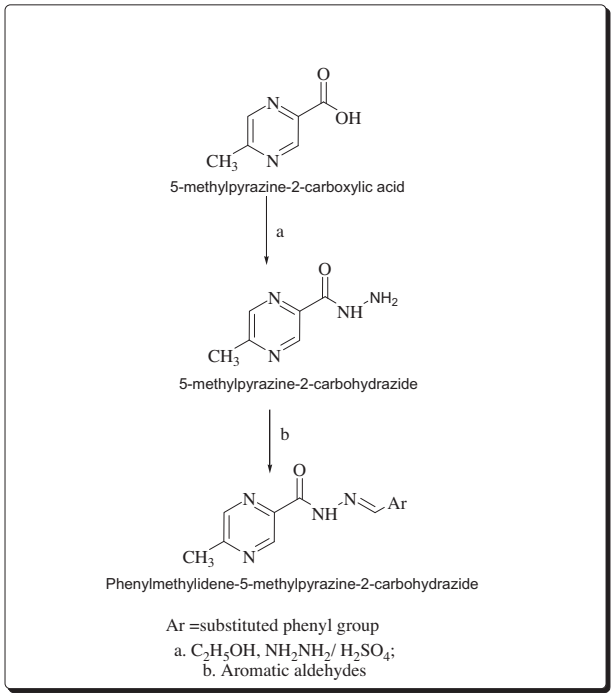
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.arabjc.2013.04.001



Scheme 1 PM series.

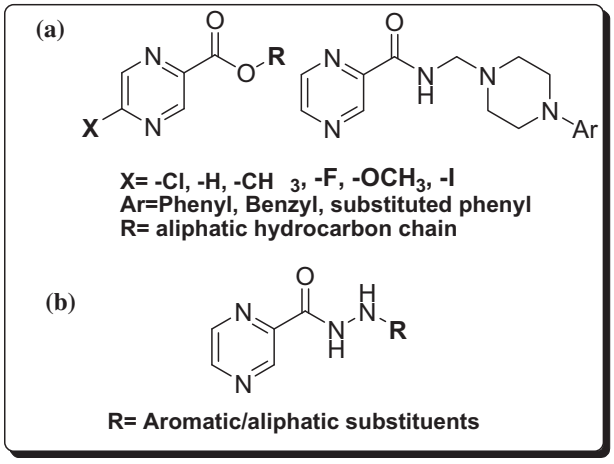


Figure 1 Pyrazine derivatives reported for anti-tubercular activity.

of synthetic methodology and search on new compounds related to 'Pyrazine derivatives' which are known for their anti-tubercular activity (Fig. 1a) Sriram et al., 2006. The structural requirement for inhibition of FASI and anti-mycobacterial activity suggests the presence of a pyrazine ring with an acyl moiety (Zimhony et al., 2007).

In the present study, pyrazinoic acid hydrazides (Fig. 1b) (Miniyar and Bhat, 1999) which are active against *Mycobacterium tuberculosis*, an attempt has been made to condense various substituted aromatic aldehydes to explore the possibilities of their activity against *M. tuberculosis* (Vergara, 2009).

2. Results and discussion

2.1. Chemistry

The synthesis of PM series compounds involved a three-step process, in the first step 5-methylpyrazinoic acid was concerted into 5-methylethylpyrazinoate in the presence of ethanol and catalytic amount of Conc. H_2SO_4. The obtained 5-methylethylpyrazinoate was converted into 5-methylpyrazinoic acid hydrazide by using hydrazine hydrate (99%). Finally, the 5-methylpyrazinoic acid hydrazide was condensed with different substituted aromatic aldehydes in the presence of ethanol yielding various substituted Phenylmethylidene-5-methylpyrazine-2-carbohydrazide derivatives (see Scheme 1) (Table 1).

2.2. Biological activity

The anti-TB activity of PM series against *M. tuberculosis* H37RV strain was performed by the Middlebrooke 7H-9 method. Compound PM 14 (5-methyl-N′-{[4-dimethylamino) phenyl] methylidene} pyrazine-2-carbohydrazide) was found to be more promising against *M. tuberculosis* amongst the compounds tested at concentration 10–50 μg/mL, whereas compounds PM 5–7, 11–13 (Table 2) were moderately active between 25 and 50 μg/mL concentration as compared with the standard anti-TB agents and the −log MIC activity was found in the range of 1.011–1.274. The most active compound PM 14 showed maximum activity 1.409 and was found to be more sensitive than INH (1.137) and PZA (1.115) standard anti-TB agents. It is clearly observed that 5-methyl substitution has a certain role in the activity result.

2.3. Acute toxicity studies

The lethal dose (LD50) value of PM 14 anti-TB compounds from the PM series was determined in albino rats as per OECD (Organization of Economic and Co-operation Development) guidelines. As there were no signs of mortality or any clinical abnormality, the compounds were categorized under GHS (Globally Harmonized System) Category 5, > 2000–5000 mg/kg body weight, with LD50 cut-off at 2500 mg/kg body weight.

2.4. 2D QSAR study

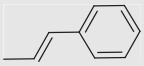
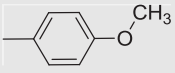
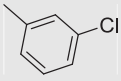
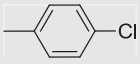
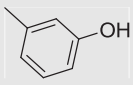
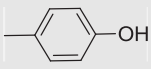
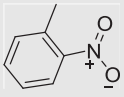
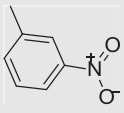
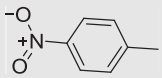
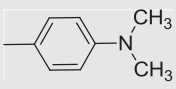
The 2-D QSAR experiment was performed using the software, TSAR 3.3, Accelerys, USA on the synthesized compounds. Multiple linear regression analysis was performed on the present series keeping biological activity as dependant variables. Various regression equations were generated, but the equation, which showed good correlation between the physicochemical property and their biological activity, was selected.

The 2-D QSAR experiment (Fig. 2) illustrates the design of PM series of compounds as potential anti-tubercular scaffolds that can be further optimized to improve the activity.

The best QSAR equation that shows statistically significance parameter is shown below.

$$\log BA = -0.50514621(\text{Inertia Moment 3 Length whole molecule}) + 0.24788234(\text{Kier Chi 0 atoms Index whole molecule}) - 0.94991529$$

Table 1 Physical data for compounds in **PM** series.

Molecule code	Ar	Molecular formula	[a]M. P.	%Yield
PM1		$C_{13}H_{12}N_4O$	235–236	65
PM2		$C_{15}H_{14}N_4O$	172–174	45
PM3		$C_{11}H_{10}N_4O_2$	224–225	68
PM4		$C_{14}H_{14}N_4O_2$	178–180	54
PM5		$C_{13}H_{11}ClN_4O$	156–157	55
PM6		$C_{13}H_{11}ClN_4O$	226–227	50
PM7		$C_{13}H_{11}ClN_4O$	90–91	46
PM8		$C_{13}H_{11}N_5O_3$	98–90	52
PM9		$C_{13}H_{11}N_5O_3$	114–115	42
PM10		$C_{13}H_{11}N_5O_3$	132–134	56
PM11		$C_{13}H_{12}N_4O_2$	217–218	67
PM12		$C_{13}H_{12}N_4O_2$	248–249	57
PM13		$C_{13}H_{12}N_4O_2$	266–268	66
PM14		$C_{15}H_{17}N_5O$	268–270	71

[a] Melting point °C.

Table 2 Anti-tubercular activity of PM series derivatives.

Compound Code	Concentration (μg/mL)			Activity -log (MIC)[a]
	10	25	50	
PM1	R	R	R	NA
PM2	R	R	R	NA
PM3	R	R	R	NA
PM4	R	R	R	NA
PM5	R	S	S	1.041
PM6	R	S	S	1.061
PM7	R	S	S	1.118
PM8	R	R	R	NA
PM9	R	R	R	NA
PM10	R	R	R	NA
PM11	R	S	S	1.011
PM12	R	S	S	1.057
PM13	R	S	S	1.274
PM14	S	S	S	1.409
STM	S	S	S	1.889
INH	S	S	S	1.137
PZA	S	S	S	1.115

[a] MIC is the minimum inhibitory concentration against *M. tuberculosis* H37Rv by Middlebrook 7H-9 broth; *R* = resistant *S* = sensitive NA = no activity.

R	r^2	r^2cv	*F*	*s*
0.8500	0.7225	0.6390	14.32	0.1428

The above equation revealed that the following two parameters contributed for the potential anti-TB activity. Kier chi indices are the connectivity indices, which define the position of the substituents on the aromatic ring system present in the molecule. Consider the examples of most active molecules PM 7, PM 13, PM 14 having Cl, OH and $N(CH_3)_2$ groups at para positions respectively. However, the CH_3 substitution on the pyrazine ring at the 5th position increased the anti-TB activity. Kierchi is positively correlated in the present QSAR equation stating that the molecules having branched chain substituents (such as CH_3, C_2H_5) may have higher anti-TB activity.

Moment of inertia is a structural descriptor negatively correlated with biological activity, which states that the electron withdrawing groups present on the aromatic ring condensed to pyrazine carbohydrazide nucleus will be responsible for decrease in the biological activity. The compounds PM 5, PM 6, PM 7 contain electronegative atom (Cl) and have showed decreased biological activity. In short the presence of electronegative atom on aromatic ring may be responsible for reduction in the biological activity.

In conclusion, present QSAR study on substituted 5-methylpyrazine-2-carbohydrazide series revealed that the above two parameters viz., Kier chi indices contributed positively, whereas moment of inertia contributed negatively for anti-TB activity.

It can be summarized that the synthesized compounds which showed activity, inhibit and inactivate the growth of *M. tuberculosis* at lowest concentration compared with the parent nucleus pyrazinamide and these may be less toxic.

The details of 2D QSAR experiment are given in Table 3.

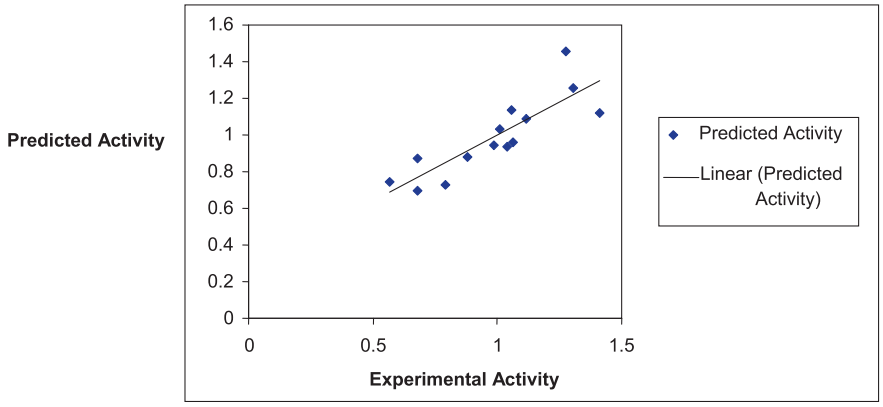


Figure 2 Predicted activity v/s experimental activity by 2-D QSAR.

Table 3 Observed v/s predicted biological (anti-tubercular) activity by 2-D QSAR.

Comp. code	Observed activity	Predicted activity	Residual value
PM 5	1.041	0.935	0.106
PM 6	1.061	0.963	0.098
PM 7	1.118	1.084	0.034
PM 11	1.011	1.119	−0.108
PM 12	1.057	1.033	0.024
PM 13	1.274	1.136	0.138
PM 14	1.409	1.457	−0.048

3. Experimental

3.1. General

Melting points are determined in open capillaries using a Veego VMP-1 melting point apparatus and are uncorrected. λ_{max} values were determined by a JASCO 630 V Ultra-violet spectrophotometer using methanol as a solvent. The IR spectra of compounds were recorded on a JASCO FTIR 4100 series spectrometer. ^{1}H NMR spectra were recorded on Varian Mercury YH-300 at 300 MHz, in DMSO-d_6/$CDCl_3$ as a solvent and TMS as the internal standard. Peak values are shown in δ ppm. The mass spectra of representative compounds were recorded on Waters Q-ToF micro by electron spray ionization method. Progress of the reaction and purity of the compounds were confirmed by pre-coated TLC plates (Merck, 60F-254) and spots were visualized using iodine vapor or UV light. The compounds were purified by re-crystallization using methanol/ethanol as a solvent.

3.2. Chemistry

3.2.1. Synthesis of pyrazinoic acid hydrazide [7]

5-Methylpyrazine-2-carboxylic acid (0.1 M) was dissolved in methanol (2.0 M). Few drops of conc. sulfuric acid were added and refluxed for a period of 24 h. Hydrazine hydrate 100% (3 M) was added to it and refluxed for a period of 8 h. The product was concentrated under reduced pressure and cooled. The solid obtained was washed with cold water and crystallized from ethanol (95%) with 87% yield.

3.2.2. Synthesis of substituted phenylmethylidene-5-methylpyrazine-2-carbohydrazide

A solution of aromatic/substitutes aldehydes (0.05 M) in ethanol was added to a solution of 5-methylpyrazine-2-carbohydrazide (0.05 M) in 10 mL ethanol. The mixture was refluxed for 4 h. After cooling the mixture, the precipitate was filtered, dried and recrystallized from aq. ethanol with 45–67% yield.

Spectral data for synthesized compounds are as follows.

3.2.2.1. PM 1: 5-methyl-N′-[phenylmethylidene] pyrazine-2-carbohydrazide. 65% yield; m.p. 235–236 °C; UV λ_{max} (CH_3OH):307; IR (Cm^{-}): 3292, 2922, 1680, 1487, 825, 692; ^{1}H NMR (300 MHz, $CDCl_3$): 8.28–8.34 (m, 2H, pyrazine), 7.38–7.78 (m, 5H, phenyl), 7.24 (s, 1H, =CH) 10.63 (s, 1H, N–H), 2.68 (s, 3H, CH_3); MS (ESI): 263.169 $[M + Na]^+$.

3.2.2.2. PM 2: 5-methyl-N′-[3-phenylprop-2-en-1-ylidene] pyrazine-2-carbohydrazide. 45% yield; m.p. 172–174 °C; UV λ_{max} (CH_3OH):271; IR (Cm^{-}): 3143, 3012, 1626, 1487, 785, 680; ^{1}H NMR (300 MHz, $CDCl_3$): 8.38–9.35 (m, 2H, pyrazine), 7.10–7.38 (m, 5H, phenyl), 7.51 (s, 1H, =CH) 6.90–7.09 (s, 2H, CH =CH), 10.56 (s, 1H, N–H), 2.68 (s, 3H, CH_3); MS (ESI): 289.112 $[M + Na]^+$.

3.2.2.3. PM 3: 5-methyl-N′-[furan-3-ylmethylidene] pyrazine-2-carbohydrazide. 68% yield; m.p. 224–225 °C; UV λ_{max} (CH_3OH):330; IR (Cm^{-}): 3302, 3007, 1674, 1485, 785, 740; ^{1}H NMR (300 MHz, $CDCl_3$): 8.35–8.44 (s, 2H, pyrazine), 6.47–6.85 (m, 3H, furyl), 7.50 (s, 1H, =CH) 10.54 (s, 1H, N-H), 2.66 (s, 3H, CH_3); MS (ESI): 253.197 $[M + Na]^+$.

3.2.2.4. PM 4: 5-methyl-N′-[(4-methoxyphenyl)methylidene] pyrazine-2-carbohydrazide. 54% yield; m.p. 178–180 °C; UV λ_{max} (CH_3OH):326; IR (Cm^{-}): 3288, 2926, 1678, 1508, 823; ^{1}H NMR (300 MHz, $CDCl_3$): 8.17–8.58 (m, 2H, pyrazine), 6.87–7.20 (m, 4H, phenyl), 7.69 (s, 1H, =CH), 3.84 (s, 3H, –OCH_3), 10.49 (s, 1H, N–H), 2.56 (s, 3H, CH_3); MS (ESI): 271.110 $[M + 1]^+$.

3.2.2.5. PM 5: 5-methyl-N′-[(2-chlorophenyl)methylidene] pyrazine-2-carbohydrazide. 55% yield; m.p. 156–157 °C; UV λ_{max} (CH_3OH):267; IR (Cm^{-}): 3287, 3013, 1676, 1587, 823, 779; ^{1}H NMR (300 MHz, $CDCl_3$): 8.64–9.07 (m, 3H, pyra-

zine), 7.21–7.38 (m, 4H, phenyl), 7.80 (s, 1H, =CH), 10.75 (s, 1H, N–H), 2.66 (s, 3H, CH_3); MS (ESI): 275.181 $[M + 1]^+$.

3.2.2.6. PM 6: 5-methyl-N′-[(3-chlorophenyl)methylidene] pyrazine-2-carbohydrazide. 50% yield; m.p. 226–227 °C; UV λ_{max} (CH_3OH):272; IR (Cm^-): 3289, 2926, 1686, 1587, 835, 613; ^1H NMR (300 MHz, $CDCl_3$): 8.72–9.02 (m, 3H, pyrazine), 7.82–8.04 (m, 4H, phenyl), 7.42 (s, 1H, =CH), 10.44 (s, 1H, N–H), 2.86 (s, 3H, CH_3); MS (ESI): 275.149 $[M + 1]^+$.

3.2.2.7. PM 7: 5-methyl-N′-[(4-chlorophenyl)methylidene] pyrazine-2-carbohydrazide. 46% yield; m.p. 90–91 °C; UV λ_{max} (CH_3OH):270; IR (Cm^-): 3287, 3013, 1676, 1587, 823, 779; ^1H NMR (300 MHz, $CDCl_3$): 8.54–8.88 (m, 2H, pyrazine), 7.40–7.98 (m, 4H, phenyl), 7.15 (s, 1H, =CH), 9.85 (s, 1H, N-H), 2.86(s, 3H, CH_3); MS (ESI): 275.127 $[M + 1]^+$.

3.2.2.8. PM 8: 5-methyl-N′-[(2-nitrophenyl)methylidene] pyrazine-2-carbohydrazide. 52% yield; m.p. 98–99 °C; UV λ_{max} (CH_3OH):263; IR (Cm^-): 3317, 2930, 1668, 1570, 1606, 700; ^1H NMR (300 MHz, $CDCl_3$): 8.39–8.80 (m, 2H, pyrazine), 7.55–7.92 (m, 4H, phenyl), 7.20 (s, 1H, =CH), 10.83 (s, 1H, N–H), 2.69 (s, 3H, CH_3); MS (ESI): 308.112 $[M + Na]^+$.

3.2.2.9. PM 9: 5-methyl-N′-[(3-nitrophenyl)methylidene] pyrazine-2-carbohydrazide. 42% yield; m.p. 114–115 °C; UV λ_{max} (CH_3OH):269; IR (Cm^-): 3307, 2910, 1658, 1521, 1606, 704; ^1H NMR (300 MHz, $CDCl_3$): 8.10–8.38 (m, 3H, pyrazine), 7.62–7.90 (m, 4H, phenyl), 7.22 (s, 1H, =CH), 10.38 (s, 1H, N–H), 2.38 (s, 3H, CH_3); MS (ESI): 308.146 $[M + 1]^+$.

3.2.2.10. PM 10: 5-methyl-N′-[(4-nitrophenyl)methylidene] pyrazine-2-carbohydrazide. 56% yield; m.p. 132–134 °C; UV λ_{max} (CH_3OH):236; IR (Cm^-): 3223, 3067, 1630, 1597, 1577, 694; ^1H NMR (300 MHz, $CDCl_3$): 8.04–8.20 (m, 3H, pyrazine), 7.66–7.80 (m, 4H, phenyl), 7.20 (s, 1H, =CH), 9.96 (s, 1H, N–H), 2.44 (s, 3H, CH_3); MS (ESI): 308.134 $[M + 1]^+$.

3.2.2.11. PM11: 5-methyl-N′-[(2-hydroxyphenyl)methylidene]pyrazine-2-carbohydrazide. 67% yield; m.p. 217–218 °C; UV λ_{max} (CH_3OH):370; IR (Cm^-): 3422, 3322, 3007, 1674, 1583, 692; ^1H NMR (300 MHz, $CDCl_3$): 8.42–8.92 (m, 3H, pyrazine), 7.20–7.60 (m, 4H, phenyl), 7.10 (s, 1H, =CH), 10.66 (s, 1H, N–H), 11.36 (s, 1H, OH), 2.80 (s, 3H, CH_3); MS (ESI): 257.219 $[M + 1]^+$.

3.2.2.12. PM12: 5-methyl-N′-[(3-hydroxyphenyl)methylidene]pyrazine-2-carbohydrazide. 57% yield; m.p. 248–249 °C; UV λ_{max} (CH_3OH):345; IR (Cm^-): 3483, 3308, 3024, 1676, 1608, 827; ^1H NMR (300 MHz, $CDCl_3$): 8.66–8.96 (m, 3H, pyrazine), 7.60–7.88 (m, 4H, phenyl), 7.40 (s, 1H, =CH), 12.36 (s, 1H, OH) 10.36 (s, 1H, N–H) 2.40 (s, 3H, CH_3); MS (ESI): 257.288 $[M + 1]^+$.

3.2.2.13. PM13: 5-methyl-N′-[(4-hydroxyphenyl)methylidene]pyrazine-2-carbohydrazide. 66% yield; m.p. 266–268 °C; UV λ_{max} (CH_3OH):345; IR (Cm^-): 3432, 3261, 3024, 1623, 1597, 732; ^1H NMR (300 MHz, $CDCl_3$): 8.53–8.64 (m, 3H, pyrazine), 7.52–7.68 (m, 4H, phenyl), 6.85 (s, 1H, =CH), 11.84 (s, 1H, OH) 10.07 (s, 1H, N–H) 2.50 (s, 3H, CH_3); MS (ESI): 257.227 $[M + 1]^+$.

3.2.2.14. PM14: 5-methyl-N′-{[4-dimethylamino)phenyl]methylidene} pyrazine-2-carbohydrazide. 71% yield; m.p. 268–270 °C; UV λ_{max} (CH_3OH):392; IR (Cm^-): 3300, 2924, 1680, 1406, 1601, 812; ^1H NMR (300 MHz, $CDCl_3$): 8.10–8.20 (m, 3H, pyrazine), 7.38–7.78 (m, 4H, phenyl), 7.12 (s, 1H, =CH), 10.20 (s, 1H, N–H), 3.15 (s, 6H, CH_3), 2.40 (s, 3H, CH_3); MS (ESI): 284.316 $[M + 1]^+$.

3.2.3. Biological activity

All synthesized molecules are tested for anti-tubercular activity using the Middle brook 7H-9 broth dilution method (Table 2) Goto et al., 1981; Villanova, 1994 and standard stain of *M. Tuberculosis* $H_{37}R_v$ (ATCC 27294).

The basal medium is prepared according to manufacturer's instructions and sterilized by autoclaving. 4.5 mL of broth is poured into each one of the sterile bottles. To this, 0.5 mL of ADC supplement contains catalase, detxrose, and bovine serum albumin fraction V. A stock solution of the compound is prepared (10 mg/mL) and an appropriate amount of solution is transferred to media bottles to achieve final concentrations of 10, 20 and 30 µg/mL. Finally, 10 µg suspension of *M. Tuberculosis* $H_{37}R_v$ strain (100000 organisms/mL adjusted by McFarland's turbidity standard) is transferred to each of the tubes and incubated at 37 °C. A growth control without compound and drug controls is also set up. The bottles are inspected for growth twice a week for a period of three weeks. The appearance of turbidity is considered as growth and indicates resistance to the compound. The growth is also confirmed by making a smear from each bottle and performing a Zeil-Nelson stain.

3.2.4. 2D QSAR study

All computational molecules were drawn in ChemDraw Ultra 8.0 and 2-D QSAR experiment was performed using the software, TSAR 3.3, Accelerys, USA on the synthesized compounds. Multiple linear regression analysis was performed on the present series keeping biological activity as dependant variables. Various regression equations were generated, but the equation, which showed good correlation between the physicochemical property and biological activity, was selected.

3.2.5. External validation

Predicted R^2 value is calculated using Equation 1, where y_i and y^i are the actual and predicted activities of the i^{th} molecule, respectively and y_{mean} is the average activity of all molecules.

Acknowledgement

We are thankful to Prof. M. N. Navale, President, Sinhgad Technical Education Society, Pune for constant encouragement and providing infrastructure. We are thankful to the Emcure Lab. Ltd., Pune for providing gift sample of pyrazinamide and Poona College of Pharmacy, Pune, for providing 2-D QSAR facility. We are also thankful to the Dr. K. G. Bhat,

M. M. Dental College, Belguam, for providing anti-TB activity facility.

References

Cynamon, M.H., Welch, J.T., Bergmann, K.E., 1991. J. Med. Chem. 39, 3394.
Cynamon, M.H., Klemen, S.P., Chou, T.S., Gimi, R.H., Welch, J.T., 1992. J. Med. Chem. 35, 1212.
Goto, S., Jo, K., Kawakita, T., Mitsuhashi, S., Nishino, T., Ohsawa, N., Hanami, H., 1981. Chemotherapy 29, 76.
Miniyar, P.B., Bhat, A.R., 1999. Indian J. Heterocyclic Chem. 9, 155.
Sriram, D., Yogeeswari, P., Reddy, S.P., 2006. Bioorg. Med. Chem. Lett. 16, 2113.
Trnka, L., Kuska, J., Harvel, A., 1964. Chemotherapy 9, 158.
Vergara Fatima, M.F., Lima Camilo, H., da, S., Henriques, M., de, O., Candea Andre, L.P., Lourenco Maria, C.S., Ferreira Marcelle, de L., Kaiser Carlos, R., de Souza Marcus, V.N., 2009. Eur. J. Mech. Chem. 2009 (44), 4954–4959.
Villanova, P.A., 1994. National committee for clinical laboratory standards, antimycobacterial susceptibility testing for mycobacterium tuberculosis.
World Health Organization Geneva, Switzerland, 2000. The WHO/IUATLD Global Project on Anti-Tuberculosis Drug Resistance Surveillance. WHO/CDS/TB/2000.278.
Zimhony, O., Vilcheze, C., Arai, M., Welch, J.T., Jacobs Jr., W.R., 2007. Antimicrob. Agents Chemother. 51, 751.