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Bioorganic & Medicinal Chemistry 15 (2007) 1896–1902
Bioorganic & Medicinal Chemistry

Discovery of novel phenoxyacetic acid derivatives as antimycobacterial agents

Mohamed Ashraf Ali and Mohammad Shaharyar*

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Jamia Hamdard University, Hamdard Nagar, New Delhi 110062, India

Received 8 November 2006; revised 5 January 2007; accepted 6 January 2007
Available online 10 January 2007

Abstract—A series of 2-{4-[1-amino (thioxo) methyl-5-(substituted phenyl)-4,5-dihydro-1*H*-3-pyrazolyl]-2-methoxyphenoxy}acetic acid and 2-{4-[1-carbamoyl-5-(substituted phenyl)-4,5-dihydro-1*H*-3-pyrazolyl]-2-methoxyphenoxy}acetic acid were synthesized and the in vitro activity of the synthesized compounds against *Mycobacterium tuberculosis* H37Rv (MTB) and INH-resistant *M. tuberculosis* (INHR-MTB) was studied. Among the synthesized compounds, compound (**3f**) 2{-[4-(1-carbamoyl-5-(chlorophenyl)-4,5-dihydro-1*H*-3-pyrazolyl]-2-methoxyphenoxy}acetic acid was found to be the most active against *M. tuberculosis* H37Rv (MTB) and INH-resistant *M. tuberculosis* (INHR-MTB) with minimum inhibitory concentration of 0.06 μg/ml.

1. Introduction

Mycobacterium tuberculosis is the primary cause of mortality due to an infectious disease in the world today. Mycobacteria are ubiquitous organisms that are becoming increasingly important intracellular pathogen that establishes an infection in oxygen-rich macrophage of the lung.[1] The emergence of AIDS, decline of socioeconomic standards, and a reduced emphasis on tuberculosis control programs contribute to the disease's resurgence in industrialized countries.[2] Resistance of *M. tuberculosis* strains to antimycobacterial agents is an increasing problem worldwide.[3–5] However, powerful new anti-TB drugs with new mechanism of action have not been developed in the last 40 years. In spite of severe toxicity on repeated dosing isoniazid (INH) is still considered to be a first-line drug for chemotherapy of tuberculosis.[6] Literature survey reveals pyrazoline derivatives are active against many Mycobacteria.[7–9] The current work describes the synthesis of novel pyrazoline moiety with encouraging antimycobacterial activity against *M. tuberculosis* H37Rv and INH-resistant *M. tuberculosis* (INHR-MTB). The chemistry of heterocyclic compounds has been an interesting field of study for long time. The synthesis of novel pyrazoline derivatives and investigation of their chemical and biological behavior have gained more importance in recent decades for biological, medicinal, and agricultural reason. Living organism finds difficulty in construction of N—N bonds that limits the natural abundance of compounds having such bonds. Pyrazoline and their derivatives, a class of compounds containing the N—N bond, exhibit a wide range of biological activities. The current work describes the synthesis of novel pyrazoline moiety with encouraging antimycobacterial activity against *M. tuberculosis* H37Rv and INH-resistant *M. tuberculosis* (INHR-MTB).

2. Results and discussion

2.1. Chemistry

The synthesis of phenoxyacetic acid derivatives has been carried out following the steps shown in the Scheme 1. In the initial step, chalcones (**2a**–**k**) were synthesized by condensing 2-(4-formyl-2-methoxyphenoxy)acetic acid with appropriate acetophenone in dilute ethanolic potassium hydroxide solution by Claisen–Schmidt condensation. The compounds (**3a–k**) and (**4a–k**) were synthesized by reacting chalcones with thiosemicarbazide and semicarbazide in glacial acetic acid (reaction time varies from 4 to 7 hrs). The purity of the compounds was checked by single-spot TLC, and the compounds

Keywords: Mycobacterial agents; Phenoxy acetic acid.
* Corresponding author. Tel.: +91 9899452373; fax: +91 11 26059663; e-mail: yarmsy@rediffmail.com

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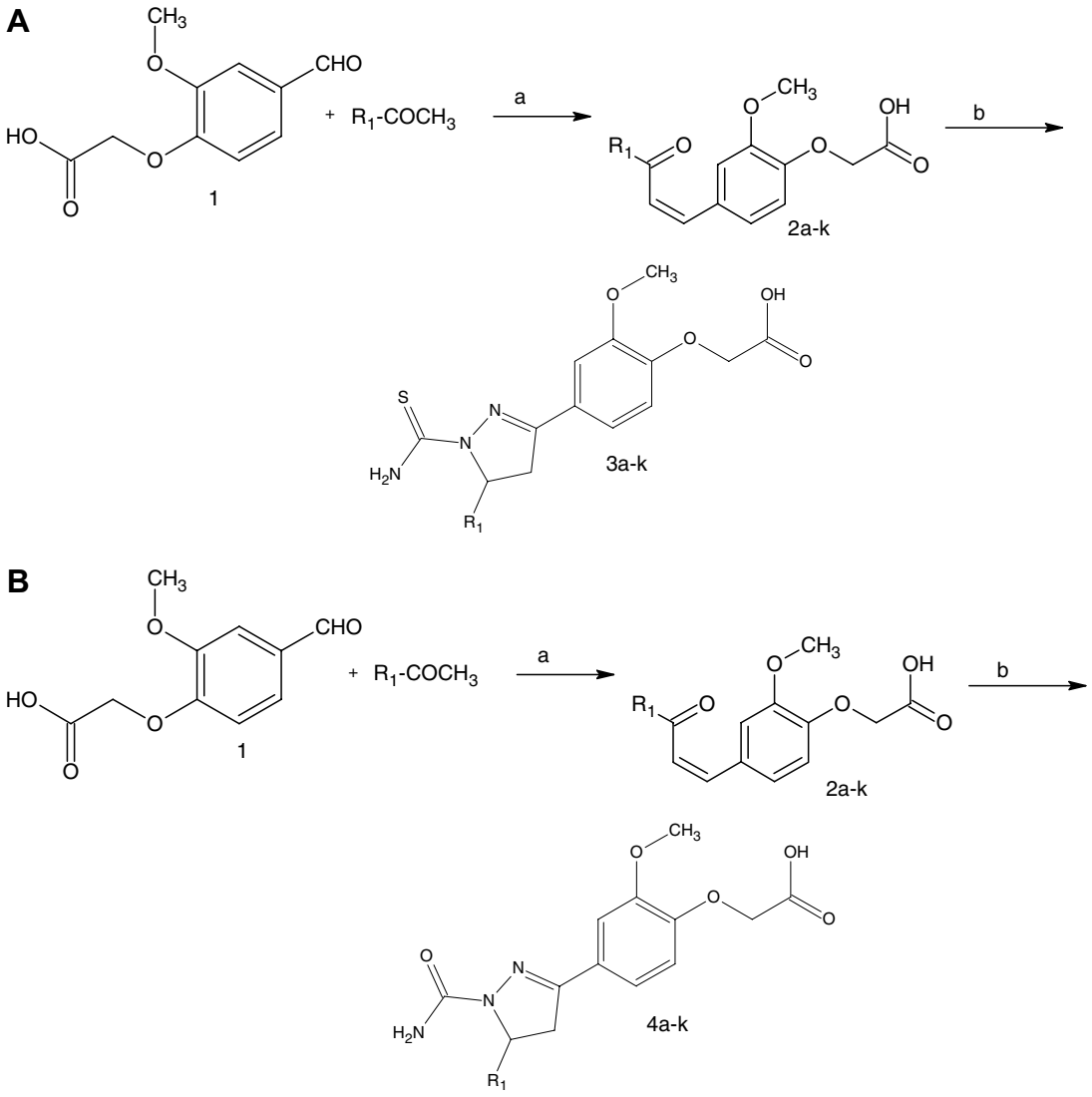
doi:10.1016/j.bmc.2007.01.006



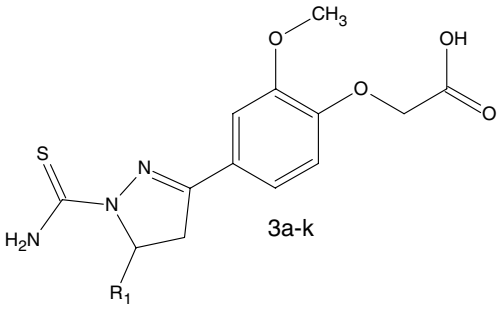
Scheme 1. Protocol for synthesis. (A) Reagents: (a) KOH/EtOH; (b) $NH_2CSNHNH_2/CH_3COOH$. (B) Reagents: (a) KOH/EtOH; (b) $NH_2CONHNH_2/CH_3COOH$.

of this study were identified by spectral data (IR, ^1H NMR, and mass). Spectral data of all the newly synthesized compounds were in full agreement with proposed structures. In general, Infra Red Spectra (IR) revealed COOH, OH, C=O, C=N, C—N and C=S peak at 3317, 3212, 1682, 1540, 1320, and 1130 cm^{-1}, respectively. In the Nuclear Magnetic Resonance Spectra (^1H NMR) the signals of the respective protons of the prepared titled compounds were verified on the basis of their chemical shifts, multiplicities, and coupling constants. The spectra showed a singlet at δ 3.0 ppm corresponding to methylene group; singlet at δ 3.4 ppm corresponding to C4 methylene group; singlet at δ 3.8 ppm corresponding to methoxy group; singlet at δ 6.0 ppm corresponding to C5 CH group; singlet at δ 7.13 ppm corresponding to NH_2 and multiplet at δ 7.2–8.3 ppm for aromatic proton; singlet at δ 8.8 ppm corresponding to OH group; singlet at δ 10.83 ppm corresponding to COOH group. The elemental analysis results were within ±0.4% of the theoretical values.

2.2. Antimycobacterial activity

All newly synthesized compounds were screened for their antimycobacterial activity against *M. tuberculosis* H37Rv using BACTEC-460 radiometric system and INH-resistant *M. tuberculosis* (INHR-MTB) using agar dilution method and MICs of the compounds are reported in Tables 1 and 2 with standard drug INH for comparison. Among the 22 newly synthesized compounds seven compounds were found to be the most active compounds with minimum inhibitory concentration of less than 1 μg/ml. The newly synthesized compounds, compound 2-{4-[1-amino(thioxo)methyl-5-(4-chlorophenyl)-4,5-dihydro-1*H*-3-pyrazolyl]-2-methoxyphenoxy}acetic acid (**3f**) was found to be the most active agent against *M. tuberculosis* H37Rv (MTB) and INH-resistant *M. tuberculosis* (INHR-MTB) with minimum inhibitory concentration of 0.06 μg/ml. Compounds with electron-withdrawing group substituted analogues showed more activity.

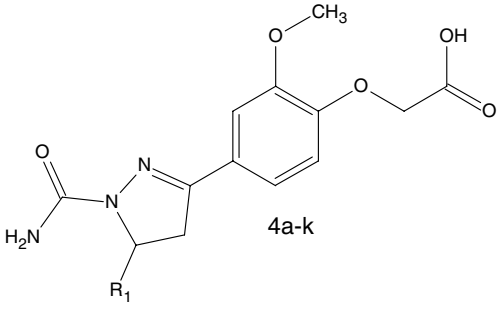
Table 1. Physical constants and antimycobacterial activity of the synthesized compounds



Compound	R	Yield (%)	Mp (°C)	Mol. formula	Mol. wt	(MIC) μg/ml	
						MTB[a]	MTB[b]
3a	Phenyl–	94	119	$C_{19}H_{19}N_3O_4S$	385.43	6.25	6.25
3b	4-Hydroxy phenyl–	78	166	$C_{19}H_{19}N_3O_5S$	401.43	0.20	0.20
3c	4-Nitro phenyl–	66	110	$C_{19}H_{18}N_4O_6S$	430.43	6.32	6.25
3d	4-Fluoro phenyl–	80	140	$C_{19}H_{18}N_3O_4SF$	403.42	0.96	0.96
3e	4-Methyl phenyl–	82	138	$C_{20}H_{21}N_3O_4S$	399.46	6.15	6.15
3f	4-Chloro phenyl–	86	186	$C_{19}H_{18}N_3O_4SCl$	419.88	0.06	0.06
3g	2-Chloro phenyl–	94	152	$C_{19}H_{18}N_3O_4SCl$	419.88	0.12	0.12
3h	4-Amino phenyl	86	146	$C_{19}H_{20}N_4O_4S$	400.45	12.5	12.5
3i	Benzyl	80	194	$C_{20}H_{21}N_3O_4S$	399.46	6.25	6.25
3j	2-Hydroxyphenyl	78	212	$C_{19}H_{19}N_3O_5S$	401.43	0.58	0.58
3k	3-Nitro phenyl–	65	112	$C_{19}H_{18}N_4O_6S$	430.43	6.25	3.12
INH	—	—	—	—	—	0.06	1.36

[a] *Mycobacterium tuberculosis* $H_{37}R_v$.
[b] INH-resistant *Mycobacterium tuberculosis*.

Table 2. Physical constants and antimycobacterial activity of the synthesized compounds



Compound	R	Yield (%)	Mp (°C)	Mol. formula	Mol. wt	(MIC) μg/ml	
						MTB[a]	MTB[b]
4a	Phenyl–	86	119	$C_{19}H_{19}N_3O_5$	369.37	6.25	6.12
4b	4-Hydroxy phenyl–	94	166	$C_{19}H_{19}N_3O_6$	385.37	1.23	1.23
4c	4-Nitro phenyl–	86	110	$C_{19}H_{18}N_4O_7$	414.37	6.25	6.25
4d	4-Fluoro phenyl–	80	140	$C_{19}H_{18}N_3O_5F$	387.36	3.16	3.25
4e	4-Methyl phenyl–	78	138	$C_{20}H_{21}N_3O_5$	383.40	4.12	6.25
4f	4-Chloro phenyl–	65	186	$C_{19}H_{18}N_3O_4Cl$	403.81	0.13	0.13
4g	2-Chloro phenyl–	66	152	$C_{20}H_{17}N_3O_4$ Cl	403.81	1.26	1.26
4h	4-Amino phenyl–	80	146	$C_{19}H_{20}N_4O_5$	384.38	5.32	5.42
4i	Benzyl	82	194	$C_{20}H_{22}N_3O_5$	383.40	6.25	6.25
4j	2-Hydroxyphenyl	86	212	$C_{19}H_{19}N_3O_6$	385.37	0.58	0.58
4k	3-Nitro phenyl–	94	112	$C_{19}H_{18}N_4O_7$	414.37	6.25	6.25
INH	—	—	—	—	—	0.06	1.36

[a] *Mycobacterium tuberculosis* $H_{37}R_v$.
[b] INH-resistant *Mycobacterium tuberculosis*.

Among the 2-{4-[1-amino(thioxo)methyl-5-(substituted phenyl)-4,5-dihydro-1*H*-3-pyrazolyl]-2-methoxyphenoxy}-acetic acid (**3a-k**) and 2-{4-(1-carbamoyl-5-(substituted phenyl)-4,5-dihydro-1*H*-3-pyrazolyl)-2-methoxyphenoxy}-acetic acid (**4a–k**) substituent with electron-withdrawing group, such as chlorine (**3f**) and (**3g**), hydroxy (**3b**) enhanced the antimycobacterial activity. However the electron withdrawing group such as 4-fluoro phenyl and

nitrophenyl-substituted analogue produced moderate inhibitory activity. On the other hand, the electron-donating group substituted analogue showed low to moderate inhibitory activity. Instead of (C=O) substitution at N-1 (C=S) group substitution in pyrazoline analogue improves the antitubercular activity. These reports clearly showed that the increases in the presence of N-1 (C=S) group substitution with 4-chlorophenyl substitution at C-5 pyrazoline (**3a–k**) derivatives **3b**, **3g**, and **3f** cause remarkable improvement in antimycobacterial activity. All the newly synthesized compounds were tested for cytotoxicity ($1C_{50}$) in VERO cells at concentrations 62.5 μg/ml or 10 times. After 72 hrs exposure, viability was assessed on the basis of cellular conversion of MTT into a formazan product using the Promega Cell Titer 96 non-radioactive cell proliferation method. Most of the active compounds were found to be non-toxic up to 62.5 μg/ml. Screening of the antitubercular activity of these novel phenoxy acetic acid derivatives identified compounds endowed with antimycobacterial activity, exhibiting MIC values less than 0.5 μg/ml. It is conceivable that derivatives showing more potency, selectivity, and low toxicity of these derivatives make them excellent leads for synthesizing novel derivatives for antimycobacterial activity, can be further modified to exhibit better potency than the standard drugs. Further studies to acquire more information about Quantitative Structure–Activity Relationships (QSAR) are in progress in our laboratory. The pyrazoline derivatives discovered in this study may provide valuable therapeutic intervention for the treatment of antitubercular diseases.

3. Experimental

All the chemicals were supplied by E. Merck (Germany) and S.D. Fine Chemicals (India). Melting points were determined by open tube capillary method and are uncorrected. Purity of the compounds was checked on thin-layer chromatography (TLC) plates (silica gel G) in the solvent system toluene–ethyl formate–formic acid (5:4:1) and benzene–methanol (8:2), the spots were located under iodine vapors or UV light. IR spectra were obtained on a Perkin-Elmer 1720 FT-IR spectrometer (KBr Pellets). ^{1}H NMR spectra were recorded on a Bruker AC 300 MHz spectrometer using TMS as internal standard in DMSO/$CDCl_3$. Mass spectra were recorded on a Bruker Esquire LCMS using ESI.

3.1. General procedure for synthesis of chalcones

2-(4-Formyl-2-methoxyphenoxy)acetic acid (0.01 mole) was dissolved in ethanol. Then, a solution of potassium hydroxide (30%, 5 ml) and suitable acetophenone in 10 ml of Petroleum ether was added to the resulting solution with continuous stirring. The resulting solution was allowed to stand overnight. After 4 hrs stirring then the solution was poured into ice-cold water and then neutralized with HCl. The solid separate was filtered off, dried, and purified by ethanol affording (**2a–k**) in 64–95% yield.

3.1.1. 2-{2-Methoxy-4-[(*Z*)-3-oxo-3-phenyl-1-propenyl]phenoxy}acetic acid (2a). IR (KBr) cm^{-1}: 3210 (OH), 1682 (C=O), 3040 (CH). ^{1}H NMR (DMSO-d_6) ppm: 10.1 (1H, s, COOH), 6.8–7.9 (7H, m, aromatic), 6.7, 7.07 (2× 1H, dd, –CH=CH, J = 9, 9 Hz), 3.7 (3H, s, OCH_3), 2.8 (2H, s, CH_2).

3.1.2. 2-{4-[(*Z*)-3-(4-hydroxyphenyl)-3-oxo-1-propenyl]-2-methoxyphenoxy}acetic acid (2b). IR (KBr) cm^{-1}: 3202 (OH), 1680 (C=O), 3042 (CH). ^{1}H NMR (DMSO-d_6) ppm: 10.1(1H, s, COOH), 8.8 (1H, s, OH), 6.8-7.82 (7H, m, aromatic), 6.7, 7.07 (2× 1H, dd, –CH=CH, J = 9, 9 Hz), 3.8 (3H, s, OCH_3), 2.7 (2H, s, CH_2).

3.1.3. 2-{2-Methoxy-4-[(*Z*)-3-(4-nitrophenyl)-3-oxo-1-propenyl]phenoxy}acetic acid (2c). IR (KBr) cm^{-1}: 3232 (OH), 1680 (C=O), 3044 (CH); ^{1}H NMR (DMSO-d_6) ppm: 10.1 (1H, s, COOH), 8.8 (1H, s, OH), 6.8–8.26 (8H, m, aromatic), 6.7, 7.07 (2× 1H, dd, –CH=CH, J = 9, 8.7 Hz), 3.8 (3H, s, OCH_3), 2.9 (2H, s, CH_2).

3.1.4. 2-{4-[(*Z*)-3-(4-fluorophenyl)-3-oxo-1-propenyl]-2-methoxyphenoxy}acetic acid (2d). IR (KBr) cm^{-1}: 3216 (OH), 1680 (C=O), 3040 (CH); ^{1}H NMR (DMSO-d_6) ppm: 10.1 (1H, s, COOH), 8.8 (1H, s, OH), 6.8–7.78 (7H, m, aromatic), 6.7, 7.07 (2× 1H, dd, –CH=CH, J = 9, 8.9 Hz), 3.8 (3H, s, OCH_3), 2.9 (2H, s, CH_2).

3.1.5. 2-{2-Methoxy-4-[(*Z*)-3-(4-methylphenyl)-3-oxo-1-propenyl]phenoxy}acetic acid (2e). IR (KBr) cm^{-1}: 3200 (OH), 1680 (C=O), 3040 (CH); ^{1}H NMR(DMSO-d_6) ppm: 10.1 (1H, s, COOH), 8.8 (1H, s, OH), 6.8–7.92 (7H, m, aromatic), 6.7, 7.07 (2× 1H, dd, –CH=CH, J = 9, 9 Hz), 3.9 (3H, s, OCH_3), 2.2 (2H, s, CH_2), 2.4 (3H, s, CH_3).

3.1.6. 2-{4-[(*Z*)-3-(4-chlorophenyl)-3-oxo-1-propenyl]-2-methoxyphenoxy}acetic acid (2f). IR (KBr) cm^{-1}: 3200(OH), 1680 (C=O), 3040 (CH). ^{1}H NMR(DMSO-d_6 ppm): 10.1 (1H, s, COOH), 8.8 (1H, s, OH), 6.8–7.86 (7H, m, aromatic), 6.7, 7.07 (2× 1H, dd, –CH=CH, J = 9, 9 Hz), 3.8 (3H, s, OCH_3), 2.9 (2H, s, CH_2).

3.1.7. 2-{4-[(*Z*)-3-(2-chlorophenyl)-3-oxo-1-propenyl]-2-methoxyphenoxy}acetic acid (2g). IR (KBr) cm^{-1}: 3200 (OH), 1680 (C=O), 3040 (CH); ^{1}H NMR (DMSO-d_6) ppm: 10.1 (1H, s, COOH), 8.8 (1H, s, OH), 6.8–7.78 (7H, m, aromatic), 6.7, 7.07 (2× 1H, dd, –CH=CH, J = 9, 9 Hz), 3.8 (3H, s, OCH_3), 2.9 (2H, s, CH_2).

3.1.8. 2-{4-[(*Z*)-3-(4-aminophenyl)-3-oxo-1-propenyl]-2-methoxyphenoxy}acetic acid (2h). IR (KBr) cm^{-1}: 3200 (OH), 1680 (C=O), 3040 (CH); ^{1}H NMR (DMSO-d_6) ppm: 10.1 (1H, s, COOH), 8.8 (1H, s, OH), 6.6–7.23 (7H, m, aromatic), 5.12 (2H, s, NH_2), 6.7, 7.07(2× 1H, dd, –CH=CH, J = 9, 9 Hz), 3.8 (3H, s, OCH_3), 2.9 (2H, s, CH_2).

3.1.9. 2-{2-methoxy-4-[(*Z*)-3-oxo-4-phenyl-1-butenyl]phenoxy}acetic acid (2i). IR (KBr) cm^{-1}: 3200 (OH), 1680 (C=O), 3040 (CH); ^{1}H NMR (DMSO-d_6) ppm: 10.1(1H, s, COOH), 8.8 (1H, s, OH), 6.8–7.70 (7H, m,

aromatic), 5.12 (2H, s, NH_2), 6.02, 7.27 (2× 1H, dd, –CH=CH, $J = 9$, 9 Hz), 3.8 (3H, s, OCH_3), 2.9 (2H, s, CH_2), 2.4 (2H, s, CH_2).

3.1.10. 2-{4-[(*Z*)-3-(2-hydroxyphenyl)-3-oxo-1-propenyl]-2-methoxyphenoxy}acetic acid (2j). IR (KBr) cm^{-1}: 3200 (OH), 1680 (C=O), 3040 (CH); ^1H NMR(DMSO-d_6) ppm: 12.1 (1H, s, COOH), 8.8 (1H, s, OH), 6.8–7.70 (7H, m, aromatic), 5.12 (2H, s, NH_2), 6.02, 7.27 (2× 1H, dd, –CH=CH, $J = 6$, 6 Hz), 3.8 (3H, s, OCH_3), 2.9 (2H, s, CH_2), 2.4 (2H, s, CH_2).

3.1.11. 2-{2-Methoxy-4-[(*Z*)-3-(3-nitrophenyl)-3-oxo-1-propenyl] phenoxy}acetic acid (2k). IR (KBr) cm^{-1}: 3200 (OH), 1680 (C=O), 3040 (CH). ^1H NMR (DMSO-d_6 ppm): 12.1 (1H, s, COOH), 8.8 (1H, s, OH), 6.8–8.82 (7H, m, aromatic), 5.12 (2H, s, NH_2), 6.02, 7.27 (2× 1H, dd, –CH=CH, $J = 9$, 9 Hz), 3.8 (3H, s, OCH_3), 2.9 (2H, s, CH_2), 2.4 (2H, s, CH_2).

3.2. General procedure for the synthesis of 2-{4-[1-amino (thioxo) methyl-5-(substituted phenyl)-4,5-dihydro-1H-3-pyrazolyl]-2-methoxyphenoxy}acetic acid (3a–k)

To a solution of (0.01 mole) chalcones (**2a–k**) in ethanol (20 ml) was added thiosemicarbazide (0.02 mole) in glacial acetic acid and the reaction mixture was refluxed for 4 hrs. The reaction mixture was cooled and then poured onto crushed ice. Then solid mass separated out was filtered, washed with water, and purified by ethanol (**3a–k**).

3.2.1. 2-{4-[1-Amino (thioxo) methyl-5-phenyl-4,5-dihydro-1*H*-3-pyrazolyl]-2-methoxyphenoxy}acetic acid (3a). IR (KBr) cm^{-1}: 3320 (NH_2), 3307 (COOH), 1590 (C=N), 1320 (C—N) 1130 (C=S); ^1H NMR (DMSO-d_6) ppm: 10.87 (1H, s, COOH), 6.83–7.51 (8H, m, aromatic), 5.34 (2H, s, NH_2), 5.82 (1H, s, CH), 3.8 (3H, s, OCH_3), 3.3 (2H, s, CH_2, C4 methylene), 2.7 (2H, s, CH_2): ESI-MS: *m*/*z*: 385 (M^+).

3.2.2. 2-{4-[1-Amino(thioxo)methyl-5-(4-hydroxyphenyl)-4,5-dihydro-1*H*-3-pyrazolyl]-2-methoxyphenoxy}acetic acid (3b). IR (KBr) cm^{-1}: 3320 (NH_2), 3307 (COOH), 1590 (C=N), 1320 (C—N) 1130 (C=S); ^1H NMR (DMSO-d_6) ppm: 10.82 (1H, s, COOH), 9.2 (1H, s, OH), 7.21–7.51 (7H, m, aromatic), 5.4 (2H, s, NH_2), 5.8 (1H, s, CH), 3.4 (3H, s, OCH_3), 3.2 (2H, s, CH_2, C4 methylene), 2.7 (2H, s, CH_2): ESI-MS: *m*/*z*: 401 (M^+).

3.2.3. 2-{4-[1-Amino (thioxo) methyl-5-(4-nitrophenyl)-4,5-dihydro-1*H*-3-pyrazolyl]-2-methoxyphenoxy}acetic acid (3c). IR (KBr) cm^{-1}: 3320 (NH_2), 3307 (COOH), 1590 (C=N), 1320 (C—N) 1130 (C=S); ^1H NMR (DMSO-d_6) ppm: 10.87 (1H, s, COOH), 7.41–8.22 (7H, m, aromatic), 6.86 (2H, s, NH_2), 6.0 (1H, s, CH), 3.9 (3H, s, OCH_3), 3.1 (2H, s, CH_2, C4 methylene), 2.7 (2H, s, CH_2): ESI-MS: *m*/*z*: 431 (M^{+1}).

3.2.4. 2-{4-[1-Amino(thioxo)methyl-5-(4-fluorophenyl)-4,5-dihydro-1*H*-3-pyrazolyl]-2-methoxyphenoxy}acetic acid (3d). IR (KBr) cm^{-1}: 3320 (NH_2), 3307 (COOH), 1590 (C=N), 1320 (C—N) 1130 (C=S); ^1H NMR (DMSO-d_6) ppm: 10.87 (1H, s, COOH), 7.21–7.51 (7H, m, aromatic), 6.86 (2H, s, NH_2), 6.0 (1H, s, CH), 3.4 (3H, s, OCH_3), 3.0 (2H, s, CH_2, C4 methylene), 2.7 (2H, s, CH_2): ESI-MS: *m*/*z*: 403 (M^+).

3.2.5. 2-{4-[1-Amino (thioxo) methyl-5-(4-methylphenyl)-4,5-dihydro-1*H*-3-pyrazolyl]-2-methoxyphenoxy}acetic acid (3e). IR (KBr) cm^{-1}: 3320 (NH_2), 3307 (COOH), 1590 (C=N), 1320 (C—N) 1130 (C=S); ^1H NMR (DMSO-d_6) ppm: 10.87 (1H, s, COOH), 7.21–7.51 (7H, m, aromatic), 6.86 (2H, s, NH_2), 6.0 (1H, s, CH), 3.4 (3H, s, OCH_3) 3.2 (3H, s, CH_3), 2.7 (2H, s, CH_2, C4 methylene), 2.7 (2H, s, CH_2): ESI-MS: *m*/*z*: 400 (M^{+1}).

3.2.6. 2-{4-[1-Amino (thioxo) methyl-5-(4-chlorophenyl)-4,5-dihydro-1*H*-3-pyrazolyl]-2-methoxyphenoxy}acetic acid (3f). IR (KBr) cm^{-1}: 3320 (NH_2), 3307 (COOH), 1590 C=N), 1320 (C—N) 1130 (C=S), 776(C—Cl); ^1H NMR(DMSO-d_6) ppm: 10.87 (1H, s, COOH), 7.21–7.51 (7H, m, aromatic), 6.86 (2H, s, NH_2), 6.0 (1H, s, CH), 3.4 (3H, s, OCH_3), 3.1 (2H, s, CH_2, C4 methylene), 2.7 (2H, s, CH_2): ESI-MS: *m*/*z*: 419 (M^+).

3.2.7. 2-{4-[1-Amino (thioxo) methyl-5-(2-chlorophenyl)-4,5-dihydro-1*H*-3-pyrazolyl]-2-methoxyphenoxy}acetic acid (3g). IR (KBr) cm^{-1}: 3320 (NH_2), 3307 (COOH), 1590(C=N), 1320 (C—N) 1130 (C=S), 776(C—Cl); ^1H NMR (DMSO-d_6) ppm: 10.87 (1H, s, COOH), 7.21–7.51 (7H, m, aromatic), 6.86 (2H, s, NH_2), 6.0 (1H, s, CH), 3.4 (3H, s, OCH_3), 3.1 (2H, s, CH_2, C4 methylene), 2.7 (2H, s, CH_2): ESI-MS: *m*/*z*: 420 (M^{+1}).

3.2.8. 2-{4-[5-(4-Aminophenyl)-1-amino (thioxo) methyl-4,5-dihydro-1*H*-3-pyrazolyl]-2-methoxyphenoxy} acetic acid (3h). IR (KBr) cm^{-1}: 3320 (NH_2), 3307 (COOH), 1590 (C=N), 1320 (C—N) 1130 (C=S); ^1H NMR (DMSO-d_6) ppm: 10.87 (1H, s, COOH), 7.21–7.51 (7H, m, aromatic), 6.86 (2H, s, NH_2), 6.82 (2H, s, NH_2) 6.0 (1H, s, CH), 3.4 (3H, s, OCH_3), 3.1 (2H, s, CH_2, C4 methylene), 2.7 (2H, s, CH_2): ESI-MS: *m*/*z*: 401 (M^{+1}).

3.2.9. 2-{4-[1-Amino (thioxo) methyl-5-benzyl-4,5-dihydro-1*H*-3-pyrazolyl]-2-methoxyphenoxy}acetic acid (3i). IR (KBr) cm^{-1}: 3320 (NH_2), 3307 (COOH), 1590 (C=N), 1320 (C—N) 1130 (C=S); ^1H NMR (DMSO-d_6) ppm: 10.87 (1H, s, COOH), 7.21–7.51 (7H, m, aromatic), 6.86 (2H, s, NH_2), 6.0 (1H, s, CH), 3.4 (3H, s, OCH_3), 3.1 (2H, s, CH_2, C4 methylene), 2.7 (2H, s, CH_2), 2.1(2H, s, benzylCH_2): ESI-MS: *m*/*z*: 399 (M^+).

3.2.10. 2-{4-[1-Amino (thioxo) methyl-5-(2-hydroxyphenyl)-4,5-dihydro-1*H*-3-pyrazolyl]-2-methoxyphenoxy}-acetic acid (3j). IR (KBr) cm^{-1}: 3320 (NH_2), 3307 (COOH), 1590 (C=N), 1320 (C—N) 1130 (C=S); ^1H NMR (DMSO-d_6) ppm: 10.87 (1H, s, COOH), 9.4 (1H, s, OH), 7.21–7.51 (7H, m, aromatic), 6.86 (2H, s, NH_2), 6.0 (1H, s, CH), 3.4 (3H, s, OCH_3), 3.1 (2H, s, CH_2, C4 methylene), 2.7 (2H, s, CH_2): ESI-MS: *m*/*z*: 401 (M^+).

3.2.11. 2-{4-[1-Amino (thioxo) methyl-5-(3-nitrophenyl)-4,5-dihydro-1*H*-3-pyrazolyl]-2-methoxyphenoxy}acetic acid (3k). IR (KBr) cm^{-1}: 3320 (NH_2), 3307 (COOH), 1590 (C=N), 1320 (C—N) 1130 (C=S); ^1H NMR

(DMSO-d_6) ppm: 10.87 (1H, s, COOH), 7.21–8.34 (7H, m, aromatic), 6.86 (2H, s, NH_2), 6.0 (1H, s, CH), 3.4 (3H, s, OCH_3), 3.1(2H, s, CH_2, C4 methylene), 2.7 (2H, s, CH_2): ESI-MS: *m*/*z*: 430 (M^+).

3.3. General procedure for synthesis of 2-{4-(1-carbamoyl-5-(substituted phenyl)-4,5-dihydro-1*H*-3-pyrazolyl)-2-methoxyphenoxy}acetic acid (4a–k)

To a solution of (0.01 mole) chalcone (**2a–k**) in ethanol (20 ml) was added semicarbazide (0.02 mole) in glacial acetic acid and the reaction mixture was refluxed for 4 hrs.The reaction mixture was cooled and then poured onto crushed ice. Then solid mass separated out was filtered, washed with water, and purified by ethanol (**4a–k**).

3.3.1. 2-{4-(1-carbamoyl-5-phenyl-4,5-dihydro-1*H*-3-pyrazolyl)-2-methoxyphenoxy}acetic acid (4a). IR (KBr) cm^{-1}: 3320 (NH_2), 3307 (COOH), 1590 (C=N), 1320 (C—N); ^{1}H NMR (DMSO-d_6) ppm: 10.87 (1H, s, COOH), 6.83–7.51 (8H, m, aromatic), 6.12 (2H, s, NH_2), 4.81 (1H, s, CH), 3.8 (3H, s, OCH_3), 3.3 (2H, s, CH_2, C4 methylene), 2.7 (2H, s, CH_2): ESI-MS: *m*/*z*: 369 (M^+).

3.3.2. 2-{4-[1-Carbamoyl-5-(4-hydroxyphenyl)-4,5-dihydro-1*H*-3-pyrazolyl]-2-methoxyphenoxy}acetic acid (4b). IR (KBr) cm^{-1}: 3322 (NH_2), 3307 (COOH), 1590 (C=N), 1320 (C—N); ^{1}H NMR (DMSO-d_6) ppm: 10.82 (1H, s, COOH), 9.2 (1H, s, OH), 7.21–7.51(7H, m, aromatic), 5.98 (2H, s, NH_2), 4.85 (1H, s,CH), 3.4 (3H, s, OCH_3), 3.2(2H, s, CH_2, C4 methylene), 2.7 (2H, s, CH_2): ESI-MS: *m*/*z*: 385 (M^+).

3.3.3. 2-{4-[1-Carbamoyl-5-(4-nitrophenyl)-4,5-dihydro-1*H*-3-pyrazolyl]-2-methoxyphenoxy}acetic acid (4c). IR (KBr) cm^{-1}: 3320 (NH_2), 3307 (COOH), 1590 (C=N), 1320 (C—N); ***^{1}H NMR (DMSO-d_6) ppm***: 10.84 (1H, s, COOH), 7.41–8.22 (7H, m, aromatic), 6.14 (2H, s, NH_2), 4.86 (1H, s, CH), 3.9 (3H, s, OCH_3), 3.1 (2H, s, CH_2, C4 methylene), 2.7 (2H, s, CH_2): ESI-MS: *m*/*z*: 415 (M^{+1}).

3.3.4. 2-{4-[1-Carbamoyl-5-(4-fluorophenyl)-4,5-dihydro-1*H*-3-pyrazolyl]-2-methoxyphenoxy}acetic acid (4d). IR (KBr) cm^{-1}: 3310 (NH_2), 3317 (COOH), 1590 (C=N), 1320 (C—N); ^{1}H NMR (DMSO-d_6) ppm: 10.85 (1H, s, COOH), 7.21–7.51 (7H, m, aromatic), 6.14 (2H, s, NH_2), 4.84 (1H, s, CH), 3.4 (3H, s, OCH_3), 3.0 (2H, s, CH_2, C4 methylene), 2.7 (2H, s, CH_2): ESI-MS: *m*/*z*: 387 (M^+).

3.3.5. 2-{4-[1-Carbamoyl-5-(4-methylphenyl)-4,5-dihydro-1*H*-3-pyrazolyl]-2-methoxyphenoxy}acetic acid (4e). IR (KBr) cm^{-1}: 3320 (NH_2), 3307 (COOH), 1590 (C=N), 1320 (C—N); ^{1}H NMR (DMSO-d_6) ppm: 10.87 (1H, s, COOH), 7.21–7.51 (7H, m, aromatic), 6.00 (2H, s, NH_2), 4.81 (1H, s, CH), 3.4 (3H, s, OCH_3), 3.2 (3H, s, CH_3), 2.7 (2H, s, CH_2, C4 methylene), 2.7 (2H, s, CH_2): ESI-MS: *m*/*z*: 384 (M^{+1}).

3.3.6. 2-{4-[1-Carbamoyl-5-(4-chlorophenyl)-4,5-dihydro-1*H*-3-pyrazolyl]-2-methoxyphenoxy}acetic acid (4f). IR (KBr) cm^{-1}: 3320 (NH_2), 3307 (COOH), 1590 (C=N), 1320 (C—N), 776(C—Cl); ^{1}H NMR (DMSO-d_6) ppm: 10.87 (1H, s, COOH), 7.21–7.51 (7H, m, aromatic), 6.21 (2H, s, NH_2), 4.87 (1H, s, CH), 3.4 (3H, s, OCH_3), 3.1 (2H, s, CH_2, C4 methylene), 2.7 (2H, s, CH_2): ESI-MS: *m*/*z*: 403 (M^+).

3.3.7. 2-{4-[1-Carbamoyl-5-(2-chlorophenyl)-4,5-dihydro-1*H*-3-pyrazolyl]-2-methoxyphenoxy}acetic acid (4g). IR (KBr) cm^{-1}: 3320 (NH_2), 3307 (COOH), 1590 (C=N), 1320 (C—N), 776(C—Cl); ^{1}H NMR (DMSO-d_6) ppm: 10.87 (1H, s, COOH), 7.21–7.51 (7H, m, aromatic), 6.12 (2H, s, NH_2), 4.83 (1H, s, CH), 3.4 (3H, s, OCH_3), 3.1 (2H, s, CH_2, C4 methylene), 2.7 (2H, s, CH_2): ESI-MS: *m*/*z*: 403 (M^+).

3.3.8. 2-{4-[5-(4-Aminophenyl)-1-carbamoyl-4,5-dihydro-1 H-3-pyrazolyl]-2-methoxy phenox y}acetic acid (4 h). IR (KBr) cm^{-1}: 3320 (NH_2), 3307 (COOH), 1590 (C=N), 1320 (C—N); ^{1}H NMR (DMSO-d_6)ppm: 10.87 (1 H, s, COOH), 7.21–7.51 (7H, m, aromatic), 6.12 (2H, s, 2× NH_2), 6.0 (1H, s, CH), 3.4 (3H, s, OCH_3), 3.1 (2H, s, CH_2, C4 methylene), 2.7 (2H, s, CH_2): ESI-MS: *m*/*z*: 385 (M^{+1}).

3.3.9. 2-{4-(5-Benzyl-1-carbamoyl-4,5-dihydro-1*H*-3-pyrazolyl)-2-methoxyphenoxy}acetic acid (4i). IR (KBr) cm^{-1}: 3320 (NH_2), 3307 (COOH), 590 (C=N), 1320 (C—N); ^{1}H NMR (DMSO-d_6) ppm: 10.87 (1H, s, COOH), 7.21–7.51 (7H, m, aromatic), 6.14 (2H, s, NH_2), 4.83 (1H, s, CH), 3.4 (3H, s, OCH_3), 3.1 (2H, s, CH_2, C4 methylene), 2.7 (2H, s, CH_2), 2.1 (2H, s, benzylCH_2): ESI-MS: *m*/*z*: 383 (M^+).

3.3.10. 2-{4-[1-Carbamoyl-5-(2-hydroxyphenyl)-4,5-dihydro-1*H*-3-pyrazolyl]-2-methoxyphenoxy}acetic acid (4j). IR (KBr) cm^{-1}: 3310 (NH_2), 3317 (COOH), 15,800 (C=N), 1320 (C—N); ^{1}H NMR (DMSO-d_6) ppm: 10.87 (1H, s, COOH), 9.4 (1H, s, OH), 7.21–7.51 (7H, m, aromatic), 6.16 (2H, s, NH_2), 4.83 (1H, s, CH), 3.4 (3H, s, OCH_3), 3.1 (2H, s, CH_2, C4 methylene), 2.7 (2H, s, CH_2): ESI-MS: *m*/*z*: 385 (M^+).

3.3.11. 2-{4-[1-Carbamoyl-5-(3-nitrophenyl)-4,5-dihydro-1*H*-3-pyrazolyl]-2-methoxyphenoxy}acetic acid (4k). IR (KBr) cm^{-1}: 3320 (NH_2), 3307 (COOH), 1590 (C=N), 1320 (C—N); ^{1}H NMR(DMSO-d_6) ppm: 10.82 (1H, s, COOH), 7.21–8.34 (7H, m, aromatic), 6.16 (2H, s, NH_2), 4.86 (1H, s, CH), 3.4 (3H, s, OCH_3), 3.1 (2H, s, CH_2, C4 methylene), 2.7 (2H, s, CH_2): ESI-MS: *m*/*z*: 414 (M^+).

3.4. Biology

The primary screening was conducted at a concentration of 6.25 μg/ml (or molar equivalent of highest molecular weight compound in a series of congeners) against *M. tuberculosis* H37Rv (ATCC27294) and INH-resistant *M. tuberculosis* in BACTEC 12B medium using the BACTEC 460 radiometric system.[10,11] Compound demonstrating at least 90% inhibition in the primary screen is re-examined at lower concentration (MIC) in broth micro dilution assay with Almar

blue. The MIC was defined as the lowest concentration inhibiting 99% of the inoculum. Concurrent with the determination of MICs, compounds were tested for cytotoxicity (IC_{50}) in VERO cells at concentration equal to and greater than the MIC for *M. tuberculosis* H37Rv and INH-resistant *M. tuberculosis*. After 72 h exposure, viability was assessed on the basis of cellular conversion of MTT into a formazan product using the Promega cell Titer 96 non-radioactive cell proliferation assay.[12]

Acknowledgments

The author (M. Shaharyar) expresses thanks to University Grant Commission, New Delhi, India, for the research award, we thank the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF), National Institute of Allergy and Infectious Diseases Southern Research Institute/GW Long Hansen's Disease Center, Colorado State University Birmingham, Alabama, USA, for the in vitro antimycobacterial screening.

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