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Synthesis of 1*H*-pyrazolo[1,2-*b*]phthalazine-5,10-dione derivatives: assessment of their antimicrobial, antituberculosis and antioxidant activity

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Abstract A new series of pyrazolo[1,2-*b*]phthalazine derivatives (**4a**–**p**) bearing the 5-aryloxypyrazole nucleus was synthesized by one-pot, three-component, base-catalyzed cyclo condensation reaction of 3-methyl-5-aryloxy-1-aryl-1*H*-pyrazole-4-carbaldehyde (**1a**–**d**), malononitrile or ethyl cyanoacetate (**2a**–**b**) and 2,3-dihydro-1,4-phthalazinedione (**3a**–**b**) in ethanol containing an eco friendly base, NaOH, in good to excellent yields. All synthesized compounds (**4a**–**p**) were duly characterized by physicochemical parameters, ^{1}H NMR, ^{13}C NMR, FT-IR and LCMS techniques. In vitro antimicrobial activity of the synthesized compounds was investigated against a representative panel of pathogenic strains. Compounds **4e**, **4g**, **4h**, **4k** and **4o** exhibited excellent antimicrobial activity compared with first line drugs. In vitro antituberculosis activity was evaluated against *Mycobacterium tuberculosis* H37Rv, and compounds **4g** and **4o** emerged as the promising antimicrobial members with better antituberculosis activity. A brine shrimp bioassay was carried out to study the in vitro cytotoxic properties

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of the synthesized compounds. In vitro antioxidant activity was evaluated by the ferric-reducing antioxidant power method. Compounds **4c**, **4d**, **4g** and **4h** showed the highest antioxidant potencies.

Keywords Aryloxypyrazole-4-carbaldehyde · Pyrazolo[1,2-*b*]phthalazine · Antituberculosis activity · Cytotoxicity · FRAP assay

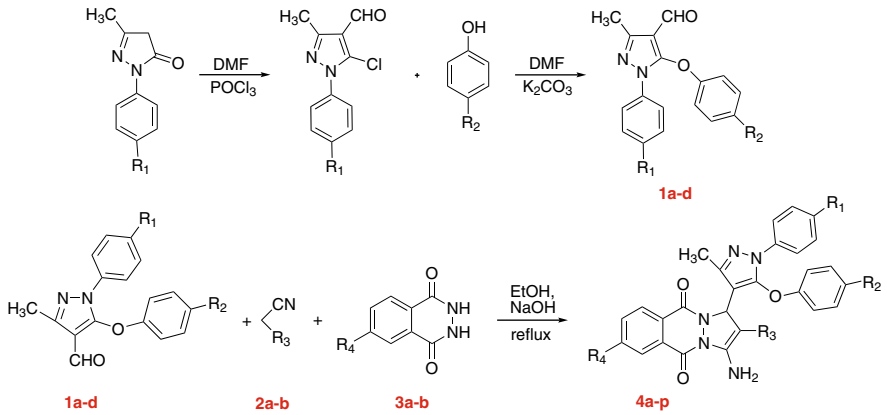
Introduction

Tuberculosis (TB) is caused predominantly by *Mycobacterium tuberculosis* bacteria (MTB), an obligate aerobic bacillum that divides at an extremely slow rate. According to the World Health Organization (WHO), in 2011, an estimated 8.7 million people died from TB [1]. The multi-drug-resistant TB strains (MDR-TBs) exhibit resistance to the front-line drugs isoniazid (INH) and rifampicin (RIF), and extensively drug resistant TB strains (XDR-TBs) exhibit resistance to second-line drugs, including fluoroquinolones, capreomycin and kanamycin [2]. These reasons make a compelling case for the urgent need for new and effective antitubercular drugs. Nitrogen-containing heterocyclic compounds are widespread in nature, and their applications in biologically active pharmaceuticals, agrochemicals, and functional materials are becoming more and more important [3–6]. Furthermore, pyrazoles are usually the core fragment of many biologically active compounds, such as Celecoxib, Viagra, Pyrazofurine and so on [7–11]. Among a large variety of N-containing heterocyclic compounds, those containing an hydrazine moiety as a "fusion site" have received considerable attention because of their pharmacological properties and clinical applications [12]. Moreover, fused phthalazines were found to possess multiple biological activities, such as antimicrobial [13], anticonvulsant [14], antifungal [15], anticancer [16] and anti-inflammatory activities [17]. Nevertheless, the development of new synthetic methods for the efficient preparation of heterocycles containing a phthalazine ring fragment is an interesting challenge. Also, multi-component reactions (MCRs) were employed as a powerful tool to synthesize diverse and complex heterocyclic compounds due to their advantages of the intrinsic atom economy, simpler procedures, structural diversity, energy savings, and reduced waste [17–19].

Despite their importance from pharmacological and synthetic points of views, recently, several elegant multi-component strategies have emerged for the synthesis of 1*H*-pyrazolo[1,2-*b*]phthalazine-5,10-dione by the cyclo condensation of phthalhydrazide, aldehydes, and malononitrile/ethyl cyanoacetate catalyzed by *p*-TSA [20], Et_3N_4 or [bmim]OH [21]. But, there was not even a single report in which 3-methyl-5-aryloxy-1-aryl-1*H*-pyrazole-4-carbaldehyde was used. Thus, in view of the biological significance of 1*H*-pyrazolo[1,2-*b*]phthalazine-5,10-dione, a modification on the 1-position of 1*H*-pyrazolo[1,2-*b*]phthalazine-5,10-dione by 3-methyl-5-phenoxy-1-phenyl-1*H*-pyrazole was undertaken to check whether it brings significant changes in the bioactivities of 1*H*-pyrazolo[1,2-*b*]phthalazine-5,10-dione derivatives. As a part of our current study in developing new antimicrobial agents via combination of two therapeutically active moieties [22–25], we report herein the

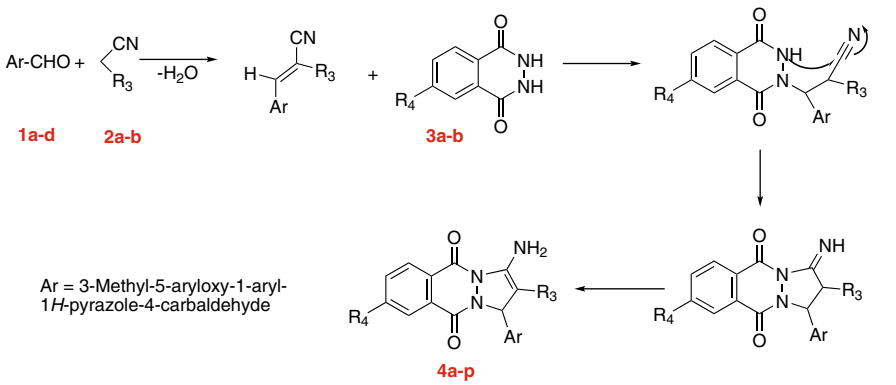
preparation 1*H*-pyrazolo[1,2-*b*]phthalazine-5,10-dione **4a**–**p** derivatives by an MCR approach. The structures of title derivatives were elucidated on the basis of FT-IR, ^{1}H NMR, ^{13}C NMR, mass spectra and elemental analysis. All these derivatives were screened for their in vitro antimicrobial activity against a representative panel of bacteria and fungi, antitubercular activity against *M. tuberculosis* H37Rv, antioxidant activity and cytotoxicity study against Artemia cysts.

In continuation of our interest on synthesizing biologically potent antimicrobials [22–25], we report herein a new series of 1*H*-Pyrazolo[1,2-*b*]phthalazine-5,10-dione derivatives **(4a**–**p**) via one-pot, three-component base-catalyzed cyclo condensation reaction of 3-methyl-5-aryloxy-1-aryl-1*H*-pyrazole-4-carbaldehyde (**1a**–**d**), malononitrile or ethyl cyanoacetate **(2a**–**b**) and 2,3-dihydro-1,4-phthalazinedione **(3a**–**b)** in ethanol containing eco friendly base NaOH in good to excellent yields (Scheme 1). The required starting material, 1-aryl-5-chloro-3-methyl-1*H*-pyrazole-4-carbaldehyde was prepared by using the procedure in the literature [26]. 1-Aryl-5-chloro-3-methyl-1*H*-pyrazole-4-carbaldehyde undergoes a nucleophilic substitution reaction with respective phenol at refluxing temperature for 4 h in the presence of a basic catalyst (K2CO3) in DMF which resulted in the required 3-methyl-5-aryloxy-1-aryl-1*H*-pyrazole-4-carbaldehyde [22, 23]. A possible mechanism for the reaction is outlined in Scheme 2. The reaction may occur via initial Knoevenagel condensation of **1a**–**d** and **2a**–**b** in the presence of NaOH base to give intermediate



Comp	R_1	R_2	R_3	R_4	Comp	R_1	R_2	R_3	R_4
4a	H	F	CN	H	**4i**	H	F	CN	NO_2
4b	H	CN	CN	H	**4j**	H	CN	CN	NO_2
4c	CH_3	F	CN	H	**4k**	CH_3	F	CN	NO_2
4d	CH_3	CN	CN	H	**4l**	CH_3	CN	CN	NO_2
4e	H	F	COOEt	H	**4m**	H	F	COOEt	NO_2
4f	H	CN	COOEt	H	**4n**	H	CN	COOEt	NO_2
4g	CH_3	F	COOEt	H	**4o**	CH_3	F	COOEt	NO_2
4h	CH_3	CN	COOEt	H	**4p**	CH_3	CN	COOEt	NO_2

Scheme 1 Synthetic pathway for the compounds **4a**–**p**



Plausible mechanistic pathway for the synthesis of 1*H*-pyrazolo[1,2-*b*]phthalazine-5,10-dione derivatives 4a-p

Scheme 2 Possible mechanistic pathway for the synthesis of compounds **4a**–**p**

heterylidenenitrile which on subsequent Michael-type addition of the 2,3-dihydro-1,4-phthalazinedione **3a**–**b**, followed by cyclization and tautomerization, affords the corresponding 1*H*-pyrazolo[1,2-*b*]phthalazine-5,10-dione derivatives **4a**–**p**.

Experimental

All reactions were performed with commercially available reagents that were used without further purification. Organic solvents were purified by standard methods and stored over molecular sieves. All melting points were taken in open capillaries and are uncorrected. Thin-layer chromatography (TLC, on aluminum plates coated with silica gel 60 F_{254}, 0.25 mm thickness, Merck) was used for monitoring the progress of all reactions, purity and homogeneity of the synthesized compounds. Elemental analysis (%C, H, N) was carried out using a Perkin-Elmer 2400 series-II elemental analyzer, and all compounds are within ±0.4 % of theory-specified values. The FTIR spectra were recorded using the potassium bromide disc on a Shimadzu FTIR 8401 spectrometer and only the characteristic peaks are reported in cm^{-1}. ^{1}H NMR and ^{13}C NMR spectra were recorded in deuterated dimethylsilane (DMSO-d_6) on a Bruker Avance 400F (MHz) spectrometer using the solvent peak as the internal standard at 400 and 100 MHz, respectively. Chemical shifts are reported in parts per million (ppm). Mass spectra were scanned on a Shimadzu LCMS 2010 spectrometer. Ampicillin, ciprofloxacin, norfloxacin, chloramphenicol, griseofulvin, nystatin, isoniazid, rifampicin and L-ascorbic acid were commercial grade.

Synthesis of compounds 4a–p

An appropriate mixture of 1*H*-pyrazole-4-carbaldehyde (**1a**–**d**; 5 mmol), malononitrile or ethylcyanoacetate (**2a**–**b**; 5 mmol), and 2,3-dihydro-1,4-phthalazinedione

(**3a**–**b**, 5 mmol) in ethanol (10 mL) containing NaOH (5 mmol, 10 mL) was heated under reflux for 3.5–4 h. On completion of reaction, monitored by TLC, the separated solid was filtered and washed well with ethanol to obtain the pure solid samples **4a**–**p**.

3-Amino-1-(5-(4-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-5,10-dioxo-5,10-dihydro-1H-pyrazolo[1,2-b]phthalazine-2-carbonitrile (**4a**) Yield: 88 %; mp 240–242 °C; IR (KBr, ν_{max}, cm^{-1}): 3395 and 3180 (asym. and sym. stretching of $-NH_2$), 2205 (C≡N stretching), 1695 (C=O stretching), 1680 (C=O stretching), 1210 (C–O–C ether stretching). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.33 (s, 3H, CH_3), δ 6.55 (s, 1H, C1H), 7.54–8.41 (m, 13H, Ar–H), 8.78 (s, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 13.02, (Ar–CH_3), 59.32 (C1), 61.32 (C2), 116.05, 124.35, 127.33, 127.90, 128.13, 128.32, 128.90, 129.40, 131.89, 132.20, 134.51, 135.41, 147.20, 151.85, 154.30, 157.87, 158.09 (Ar–C), 156.88 (C=O), 158.21 (C=O); Anal. Calcd for $C_{28}H_{19}FN_6O_3$ (506.49 g/mol): C, 66.40; H, 3.78; N, 16.59 (%); Found: C, 66.22; H, 3.62; N, 16.87 (%). MS: 506 $[M + H]^+$.

3-Amino-1-(5-(4-cyanophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-5,10-dioxo-5,10-dihydro-1H-pyrazolo[1,2-b]phthalazine-2-carbonitrile (**4b**) Yield: 85 %; mp 257–259 °C; IR (KBr, ν_{max}, cm^{-1}): 3375 and 3170 (asym. and sym. stretching of $-NH_2$), 2190 (C≡N stretching), 1680 (C=O stretching), 1665 (C=O stretching), 1195 (C–O–C ether stretching). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.54 (s, 3H, CH_3), 6.50 (s, 1H, C1H), 7.59–8.28 (m, 13H, Ar–H), 8.70 (s, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 13.05 (Ar–CH_3), 59.90 (C1), 62.07 (C2), 116.44, 117.14, 125.05, 127.17, 127.78, 128.84, 129.35, 130.13, 131.45, 132.37, 133.95, 134.56, 135.38, 137.92, 142.14, 151.71, 154.79, 156.15 (Ar–C), 157.18 (C=O), 158.25 (C=O); Anal. Calcd for $C_{29}H_{19}N_7O_3$ (513.51 g/mol): C, 67.83; H, 3.73; N, 19.09 (%); Found: C, 68.05; H, 3.89; N, 19.15 (%). MS: 513 $[M + H]^+$.

3-Amino-1-(5-(4-fluorophenoxy)-3-methyl-1-p-tolyl-1H-pyrazol-4-yl)-5,10-dioxo-5,10-dihydro-1H-pyrazolo[1,2-b]phthalazine-2-carbonitrile (**4c**) Yield: 87 %; mp 231–233 °C; IR (KBr, ν_{max}, cm^{-1}): 3395 and 3180 (asym. and sym. stretching of $-NH_2$), 2205 (C≡N stretching), 1685 (C=O stretching), 1665 (C=O stretching), 1200 (C–O–C ether stretching). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.10, 2.32 (s, 6H, 2 × CH_3), 6.53 (s, 1H, C1H), 7.35–8.34 (m, 12H, Ar–H), 8.69 (s, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 13.06, 20.80 (Ar–CH_3), 60.18 (C1), 62.50 (C2), 117.17, 124.80, 127.12, 127.40, 127.80, 128.50, 128.90, 131.16, 132.15, 133.70, 135.16, 137.17, 145.80, 151.01, 154.96, 157.13, 158.01 (Ar–C), 157.34 (C=O), 158.43 (C=O); Anal. Calcd for $C_{29}H_{21}FN_6O_3$ (520.51 g/mol): C, 66.92; H, 4.07; N, 16.15 (%); Found: C, 66.73; H, 3.90; N, 16.37 (%). MS: 520 $[M + H]^+$.

3-Amino-1-(5-(4-cyanophenoxy)-3-methyl-1-p-tolyl-1H-pyrazol-4-yl)-5,10-dioxo-5,10-dihydro-1H-pyrazolo[1,2-b]phthalazine-2-carbonitrile (**4d**) Yield: 86 %; mp 250–252 °C; IR (KBr, ν_{max}, cm^{-1}): 3380 and 3185 (asym. and sym. stretching of $-NH_2$), 2190 (C≡N stretching), 1685 (C=O stretching), 1660 (C=O stretching), 1210 (C–O–C ether stretching). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.99, 2.39 (s, 6H, 2 × CH_3), 7.82–8.35 (m, 12H, Ar–H), 8.76 (s, 2H, NH_2). ^{13}C NMR (100 MHz,

DMSO-d_6) δ: 13.00, 20.75 (Ar–CH_3), 59.23 (C1), 61.80 (C2), 116.90, 117.16, 124.60, 126.80, 127.18, 127.90, 128.17, 129.44, 131.40, 132.50, 133.31, 133.60, 134.50, 135.19, 147.17, 151.91, 154.24, 157.25 (Ar–C), 157.00 (C=O), 158.30 (C=O); Anal. Calcd for $C_{30}H_{21}N_7O_3$ (527.53 g/mol): C, 68.30; H, 4.01; N, 18.59 (%); Found: C, 67.98; H, 4.18; N, 18.80 (%). MS: 527 $[M + H]^+$.

Ethyl 3-amino-1-(5-(4-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-5,10-dioxo-5,10-dihydro-1H-pyrazolo[1,2-b]phthalazine-2-carboxylate (**4e**) Yield: 84 %; mp 219–221 °C; IR (KBr, ν_{max}, cm^{-1}): 3395 and 3300 (asym. and sym. stretching of –NH_2), 1700 (ester C=O stretching), 1670 (C=O stretching), 1650 (C=O stretching), 1195 (C–O–C ether stretching). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.07 (t, 3H, CH_3), 2.48 (s, 3H, CH_3), 3.99 (q, 2H, OCH_2), 6.56 (s, 1H, C1H), 7.61–8.40 (m, 13H, Ar–H), 8.82 (s, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 14.32 (CH_3), 13.08, (Ar–CH_3), 58.98 (C1), 63.55 (OCH_2), 82.07 (C2), 124.18, 126.85, 127.11, 127.24, 127.40, 127.75, 129.54, 131.40, 132.44, 134.08, 135.96, 146.50, 151.10, 154.90, 157.33, 158.23 (Ar–C), 156.37 (C=O), 158.55 (C=O), 164.35 (COOEt); Anal. Calcd for $C_{30}H_{24}FN_5O_5$ (553.54 g/mol): C, 65.09; H, 4.37; N, 12.65 (%); Found: C, 65.23; H, 4.08; N, 12.39 (%). MS: 553 $[M + H]^+$.

Ethyl 3-amino-1-(5-(4-cyanophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-5,10-dioxo-5,10-dihydro-1H-pyrazolo[1,2-b]phthalazine-2-carboxylate (**4f**) Yield: 82 %; mp 260–262 °C; IR (KBr, ν_{max}, cm^{-1}): 3425 and 3335 (asym. and sym. stretching of –NH_2), 2210 (C≡N stretching), 1710 (ester C=O stretching), 1675 (C=O stretching), 1650 (C=O stretching), 1190 (C–O–C ether stretching). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.09 (t, 3H, CH_3), 2.38 (s, 3H, CH_3), 3.95 (q, 2H, OCH_2), 6.55 (s, 1H, C1H), 7.64–8.29 (m, 13H, Ar–H), 8.73 (s, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 14.28 (CH_3), 13.04, (Ar–CH_3), 60.03 (C1), 63.70 (OCH_2), 81.90 (C2), 117.29, 124.80, 127.37, 127.50, 127.78, 128.93, 129.06, 130.17, 131.06, 133.72, 134.67, 135.19, 136.94, 145.22, 151.12, 153.58, 155.19 (Ar–C), 156.88 (C=O), 158.07 (C=O), 164.56 (COOEt); Anal. Calcd for $C_{31}H_{24}N_6O_5$ (560.56 g/mol): C, 66.42; H, 4.32; N, 14.99 (%); Found: C, 66.65; H, 4.18; N, 14.71 (%). MS: 560 $[M + H]^+$.

Ethyl 3-amino-1-(5-(4-fluorophenoxy)-3-methyl-1-p-tolyl-1H-pyrazol-4-yl)-5,10-dioxo-5,10-dihydro-1H-pyrazolo[1,2-b]phthalazine-2-carboxylate (**4g**) Yield: 85 %; mp 227–229 °C; IR (KBr, ν_{max}, cm^{-1}): 3465 and 3345 (asym. and sym. stretching of –NH_2), 1695 (ester C=O stretching), 1665 (C=O stretching), 1635 (C=O stretching), 1205 (C–O–C ether stretching). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.02 (t, 3H, CH_3), 2.09, 2.36 (s, 6H, 2 × CH_3), 4.01 (q, 2H, OCH_2), 6.44 (s, 1H, C1H), 7.30–8.37 (m, 12H, Ar–H), 8.46 (s, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 14.30 (CH_3), 13.10, 21.00 (Ar–CH_3), 59.88 (C1), 63.71 (OCH_2), 82.50 (C2), 123.71, 127.24, 127.57, 127.62, 128.93, 129.10, 129.36, 130.19, 132.40, 134.28, 135.20, 144.70, 149.16, 150.80, 155.56, 158.20 (Ar–C), 157.14 (C=O), 158.17 (C=O), 164.17 (COOEt); Anal. Calcd for $C_{31}H_{26}FN_5O_5$ (567.57 g/mol): C, 65.60; H, 4.62; N, 12.34 (%); Found: C, 65.78; H, 4.37; N, 12.21 (%). MS: 567 $[M + H]^+$.

Ethyl 3-amino-1-(5-(4-cyanophenoxy)-3-methyl-1-p-tolyl-1H-pyrazol-4-yl)-5,10-dioxo-5,10-dihydro-1H-pyrazolo[1,2-b]phthalazine-2-carboxylate (**4h**) Yield: 89 %; mp

270–272 °C; IR (KBr, ν_{max}, cm^{-1}): 3450 and 3340 (asym. and sym. stretching of –NH_2), 2205 (C≡N stretching), 1710 (ester C=O stretching), 1670 (C=O stretching), 1655 (C=O stretching), 1210 (C–O–C ether stretching). ^{1}H NMR (400 MHz, DMSO-d_6): 0.99 (t, 3H, CH_3), 1.95, 2.25 (s, 6H, 2 × CH_3), 3.99 (q, 2H, OCH_2), 6.57 (s, 1H, C1H), 7.80–8.35 (m, 12H, Ar–H), 8.75 (s, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 14.50 (CH_3), 13.07, 20.25 (Ar–CH_3), 59.88 (C1), 63.22 (OCH_2), 82.45 (C2), 117.23, 124.16, 124.84, 127.11, 127.55, 128.15, 128.53, 130.18, 132.40, 132.81, 133.17, 133.99, 135.28, 146.80, 151.30, 153.37 155.12 (Ar–C), 157.23 (C=O), 158.67 (C=O), 164.22 (COOEt); Anal. Calcd for $C_{32}H_{26}N_6O_5$ (574.59 g/mol): C, 66.89; H, 4.56; N, 14.63 (%); Found: C, 67.16; H, 4.82; N, 14.77 (%). MS: 574 $[M + H]^+$.

3-Amino-1-(5-(4-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-7-nitro-5,10-dioxo-5,10-dihydro-1H-pyrazolo[1,2-b]phthalazine-2-carbonitrile (**4i**) Yield: 80 %; mp 235–237 °C; IR (KBr, ν_{max}, cm^{-1}): 3400 and 3250 (asym. and sym. stretching of –NH_2), 2205 (C≡N stretching), 1685 (C=O stretching), 1670 (C=O stretching), 1195 (C–O–C ether stretching). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.44 (s, 3H, CH_3), δ 6.50 (s, 1H, C1H), 7.61–8.58 (m, 12H, Ar–H), 8.90 (s, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 13.06, (Ar–CH_3), 58.90 (C1), 61.02 (C2), 116.30, 125.75, 126.80, 127.45, 127.98, 128.19, 129.40, 129.60, 130.42, 131.14, 132.16, 134.17, 134.80, 146.40, 147.70, 151.93, 152.24, 154.70, 158.23 (Ar–C), 157.65 (C=O), 158.40 (C=O); Anal. Calcd for $C_{28}H_{18}FN_7O_5$ (551.48 g/mol): C, 60.98; H, 3.29; N, 17.78 (%); Found: C, 61.19; H, 2.96; N, 17.93 (%). MS: 551 $[M + H]^+$.

3-Amino-1-(5-(4-cyanophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-7-nitro-5,10-dioxo-5,10-dihydro-1H-pyrazolo[1,2-b]phthalazine-2-carbonitrile (**4j**) Yield: 81 %; mp 246–248 °C; IR (KBr, ν_{max}, cm^{-1}): 3385 and 3185 (asym. and sym. stretching of –NH_2), 2200 (C≡N stretching), 1680 (C=O stretching), 1660 (C=O stretching), 1185 (C–O–C ether stretching). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.31 (s, 3H, CH_3), 6.53 (s, 1H, C1H), 7.55–8.60 (m, 12H, Ar–H), 8.80 (s, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 13.01, (Ar–CH_3), 59.67 (C1), 61.49 (C2), 116.18, 117.17, 124.95, 125.64, 126.30, 127.81, 128.55, 128.80, 129.30, 131.74, 132.18, 133.16, 134.11, 134.70, 135.85, 144.14, 148.23, 151.40, 152.56, 156.81 (Ar–C), 157.46 (C=O), 158.38 (C=O); Anal. Calcd for $C_{29}H_{18}N_8O_5$ (558.50 g/mol): C, 62.36; H, 3.25; N, 20.06(%); Found: C, 62.47; H, 3.46; N, 19.87 (%). MS: 558 $[M + H]^+$.

3-Amino-1-(5-(4-fluorophenoxy)-3-methyl-1-p-tolyl-1H-pyrazol-4-yl)-7-nitro-5,10-dioxo-5,10-dihydro-1H-pyrazolo[1,2-b]phthalazine-2-carbonitrile (**4k**) Yield: 76 %; mp 254–256 °C; IR (KBr, ν_{max}, cm^{-1}): 3385 and 3200 (asym. and sym. stretching of –NH_2), 2210 (C≡N stretching), 1685 (C=O stretching), 1670 (C=O stretching), 1205 (C–O–C ether stretching). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.17, 2.39 (s, 6H, 2 × CH_3), 6.60 (s, 1H, C1H), 7.25–8.60 (m, 11H, Ar–H), 8.70 (s, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 13.15, 20.50 (Ar–CH_3), 60.00 (C1), 61.90 (C2), 116.33, 124.17, 127.21, 127.84, 128.14, 128.70, 129.20, 130.05, 131.30, 133.08, 134.15, 135.50, 137.18, 143.40, 149.80, 150.16, 151.96, 154.30, 157.90 (Ar–C), 158.80 (C=O), 158.95 (C=O); Anal. Calcd for $C_{29}H_{20}FN_7O_5$ (565.51 g/mol): C,

61.59; H, 3.56; N, 17.34 (%); Found: C, 61.81; H, 3.29; N, 17.45 (%). MS: 565 $[M + H]^+$.

3-Amino-1-(5-(4-cyanophenoxy)-3-methyl-1-p-tolyl-1H-pyrazol-4-yl)-7-nitro-5,10-dioxo-5,10-dihydro-1H-pyrazolo[1,2-b]phthalazine-2-carbonitrile (**4l**) Yield: 78 %; mp 222–224 °C; IR (KBr, ν_{max}, cm^{-1}): 3390 and 3180 (asym. and sym. stretching of $-NH_2$), 2205 (C≡N stretching), 1690 (C=O stretching), 1675 (C=O stretching), 1190 (C–O–C ether stretching). ^1H NMR (400 MHz, DMSO-d_6): δ 2.15, 2.43 (s, 6H, 2 × CH_3), δ 6.54 (s, 1H, C1H), 7.82–8.65 (m, 11H, Ar–H), 8.72 (s, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 13.11, 20.65 (Ar–CH_3), 60.12 (C1), 61.60 (C2), 116.61, 117.14, 124.20, 127.27, 127.80, 128.65, 128.84, 129.17, 130.20, 131.68, 132.80, 133.23, 133.92, 134.80, 135.30, 144.18, 147.40, 151.85, 152.04, 154.55 (Ar–C), 156.98 (C=O), 158.53 (C=O); Anal. Calcd for $C_{30}H_{20}N_8O_5$ (572.53 g/mol): C, 62.93; H, 3.52; N, 19.57 (%); Found: C, 63.11; H, 3.28; N, 19.86 (%). MS: 572 $[M + H]^+$.

Ethyl 3-amino-1-(5-(4-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-7-nitro-5,10-dioxo-5,10-dihydro-1H-pyrazolo[1,2-b]phthalazine-2-carboxylate (**4m**) Yield: 74 %; mp 264–266 °C; IR (KBr, ν_{max}, cm^{-1}): 3455 and 3330 (asym. and sym. stretching of $-NH_2$), 1715 (ester C=O stretching), 1675 (C=O stretching), 1660 (C=O stretching), 1210 (C–O–C ether stretching). ^1H NMR (400 MHz, DMSO-d_6): δ 1.05 (t, 3H, CH_3), 2.36 (s, 3H, CH_3), 3.96 (q, 2H, OCH_2), 6.48 (s, 1H, C1H), 7.47–8.55 (m, 12H, Ar–H), 8.80 (s, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 14.22 (CH_3), 13.03, (Ar–CH_3), 59.94 (C1), 63.52 (OCH_2), 82.15 (C2), 125.07, 126.84, 127.11, 127.43, 127.84, 128.53, 129.15, 129.44, 129.90, 131.20, 134.18, 135.82, 145.75, 151.26, 152.43, 154.82, 157.33, 158.40 (Ar–C), 156.50 (C=O), 158.72 (C=O), 164.19 (COOEt); Anal. Calcd for $C_{30}H_{24}FN_6O_7$ (598.54 g/mol): C, 60.20; H, 3.87; N, 14.04 (%); Found: C, 59.97; H, 4.06; N, 13.83 (%). MS: 598 $[M + H]^+$.

Ethyl 3-amino-1-(5-(4-cyanophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-7-nitro-5,10-dioxo-5,10-dihydro-1H-pyrazolo[1,2-b]phthalazine-2-carboxylate (**4n**) Yield: 72 %; mp 235–237 °C; IR (KBr, ν_{max}, cm^{-1}): 3420 and 3300 (asym. and sym. stretching of $-NH_2$), 2215 (C≡N stretching), 1700 (ester C=O stretching), 1680 (C=O stretching), 1665 (C=O stretching), 1195 (C–O–C ether stretching). ^1H NMR (400 MHz, DMSO-d_6): δ 0.99 (t, 3H, CH_3), 2.51 (s, 3H, CH_3), 3.99 (q, 2H, OCH_2), 6.57 (s, 1H, C1H), 7.68–8.69 (m, 12H, Ar–H), 8.80 (s, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 14.62 (CH_3), 13.14, (Ar–CH_3), 59.74 (C1), 63.00 (OCH_2), 82.16 (C2), 117.34, 124.24, 125.72, 127.11, 127.64, 128.75, 129.20, 129.78, 131.25, 131.82, 133.55, 134.02, 135.23, 136.42, 144.91, 145.61, 151.20, 151.35, 154.72 (Ar–C), 156.17 (C=O), 158.03 (C=O), 164.35 (COOEt); Anal. Calcd for $C_{31}H_{23}N_7O_7$ (605.56 g/mol): C, 61.49; H, 3.83; N, 16.19 (%); Found: C, 61.68; H, 3.72; N, 15.91 (%). MS: 605 $[M + H]^+$.

Ethyl 3-amino-1-(5-(4-fluorophenoxy)-3-methyl-1-p-tolyl-1H-pyrazol-4-yl)-7-nitro-5,10-dioxo-5,10-dihydro-1H-pyrazolo[1,2-b]phthalazine-2-carboxylate (**4o**) Yield: 70 %; mp 249–251 °C; IR (KBr, ν_{max}, cm^{-1}): 3470 and 3345 (asym. and sym.

stretching of $-NH_2$), 1705 (ester C=O stretching), 1685 (C=O stretching), 1650 (C=O stretching), 1200 (C–O–C ether stretching). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.03 (t, 3H, CH_3), 1.97, 2.39 (s, 6H, 2 × CH_3), 3.93 (q, 2H, OCH_2), 6.53 (s, 1H, C1H), 7.30–8.59 (m, 11H, Ar–H), 8.60 (s, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 14.50 (CH_3), 13.08, 20.20 (Ar–CH_3), 59.98 (C1), 63.55 (OCH_2), 82.55 (C2), 123.20, 127.32, 127.68, 128.12, 128.67, 129.03, 129.60, 132.27, 133.34, 134.85, 135.17, 136.79, 143.16, 149.70, 151.11, 152.30, 156.22, 158.42 (Ar–C), 156.23 (C=O), 158.39 (C=O), 164.60 (COOEt); Anal. Calcd for $C_{31}H_{25}FN_6O_7$ (612.56 g/mol): C, 60.78; H, 4.11; N, 13.72 (%); Found: C, 60.99; H, 3.89; N, 14.03 (%). MS: 612 $[M + H]^+$.

Ethyl 3-amino-1-(5-(4-cyanophenoxy)-3-methyl-1-p-tolyl-1H-pyrazol-4-yl)-7-nitro-5,10-dioxo-5,10-dihydro-1H-pyrazolo[1,2-b]phthalazine-2-carboxylate (**4p**) Yield: 72 %; mp 238–240 °C; IR (KBr, ν_{max}, cm^{-1}): 3395 and 3285 (asym. and sym. stretching of $-NH_2$), 2210 (C≡N stretching), 1705 (ester C=O stretching), 1685 (C=O stretching), 1670 (C=O stretching), 1205 (C–O–C ether stretching). ^{1}H NMR (400 MHz, DMSO-d_6): 0.96 (t, 3H, CH_3), 1.94, 2.33 (s, 6H, 2 × CH_3), 3.99 (q, 2H, OCH_2), 6.50 (s, 1H, C1H), 7.78–8.61 (m, 11H, Ar–H), 8.70 (s, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 14.45 (CH_3), 13.07, 20.75 (Ar–CH_3), 60.17 (C1), 63.34 (OCH_2), 82.60 (C2), 117.15, 124.33, 127.17, 127.70, 128.35, 128.84, 129.20, 129.65, 130.20, 131.24, 133.12, 133.60, 134.17, 135.40, 145.65, 146.17, 151.20, 152.63, 155.18 (Ar–C), 155.93 (C=O), 158.18 (C=O), 164.38 (COOEt); Anal. Calcd for $C_{32}H_{25}N_7O_7$ (619.58 g/mol): C, 62.03; H, 4.07; N, 15.82 (%); Found: C, 62.19; H, 4.23; N, 15.65 (%). MS: 619 $[M + H]^+$.

Biological assay

In vitro evaluation of antimicrobial activity

The minimum inhibitory concentrations (MICs) of synthesized compounds were carried out by a broth micro dilution method [27]. DMSO was used as the diluents to obtain the desired concentration of compounds to test upon standard bacterial strains. Serial dilutions were prepared in primary and secondary screening. The control tube containing no antibiotic was immediately sub cultured (before inoculation) by spreading a loopful evenly over a quarter plate of medium suitable for the growth of the test organism and put for incubation at 37 °C overnight. The tubes were then incubated overnight. The MIC of the control organism was checked against the accuracy of the compound concentrations. The MIC was defined as the lowest concentration of the antibiotic or test sample allowing no visible growth. All the tubes not showing visible growth (in the same manner as the control tube described above) were sub cultured and incubated overnight at 37 °C. The amount of growth of the control tube before incubation (which represents the original inoculum) was compared. Subcultures might show: a similar number of coloniesn indicating bacteriostatic condition; a reduced number of colonies, indicating a partial or slow bactericidal activity and no growth if the whole inoculum has been killed. The test must include a second set of the same dilutions inoculated with an

organism of known sensitivity. Each compound was diluted, obtaining a 2000 μg/mL concentration as a stock solution. In primary screening, 500, 250 and 200 μg/mL concentrations of the synthesized compounds were taken. The active synthesized compounds found in this primary screening were further tested in a second set of dilutions against all microorganisms. The compounds found active in primary screening were similarly diluted to obtain 100, 62.5, 50 and 25 μg/mL concentrations. The highest dilution showing at least 99 % inhibition is taken as the MIC.

In vitro evaluation of antituberculosis activity

A primary screen was conducted at 250 μg/mL against *M. tuberculosis* H37Rv by a Lowenstein-Jensen (LJ) MIC method [28] where primary 250 μg/mL dilutions of each test compound were added to liquid Lowenstein-Jensen medium and then the media were sterilized by an inspissations method. A culture of *M. tuberculosis* H37Rv growing on Lowenstein-Jensen medium was harvested in 0.85 % saline in bijou bottles. DMSO was used as a vehicle to obtain the desired concentration. The tubes were then incubated at 37 °C for 24 h followed by streaking of *M. tuberculosis* H37Rv (5 × 104 bacilli per tube). The tubes were then incubated at 37 °C. Growth of bacilli was seen after 12, 22, and, finally, 28 days of incubation. Tubes having the compounds were compared with control tubes where medium alone was incubated with *M. tuberculosis* H37Rv. The concentration at which complete inhibition of colonies occurred was taken as the active concentration of the test compound. The standard strain *M. tuberculosis* H37Rv was tested with known drugs Isoniazid and Rifampicin. The screening results are summarized as % inhibition relative to standard drugs Isoniazid and Rifampicin.

Brine shrimp lethality bioassay for evaluation of cytotoxicity

A brine shrimp lethality bioassay technique was applied for determining the general toxicity of the compounds. The in vitro lethality test has been carried out using brine shrimp eggs (Artemia cysts). Brine shrimp eggs were hatched in a shallow rectangular plastic dish (22 × 32 cm) filled with artificial seawater prepared with a commercial salt mixture and double-distilled water. An unequal partition was made in the plastic dish with the help of a perforated device. Approximately 50 mg of eggs was sprinkled into the large compartment, which was darkened while the minor compartment was opened to ordinary light. After 2 days, nauplii were collected in a pipette from the lighter side. A stock solution of the test complex was prepared in DMSO. From this stock solution, solutions were transferred to the vials to make final concentrations of 5, 10, 20, 30, 40, 50 mg/mL (dilutions were used in triplicate for each test sample, and the LC_{50} is the mean of three values) and three vials were kept as controls having DMSO only. After 2 days, when the nauplii were ready, 1 mL of seawater and 10 nauplii were added to each vial and the volume was adjusted with seawater to 2.5 mL per vial [29]. After 24 h, each vial was observed using a magnifying glass and the number of survivors in each vial was counted and noted. Data were analysed by a simple logic method to determine the LC_{50} values,

in which log× of the dose concentration of samples was plotted against the percent mortality of nauplii [30].

In vitro evaluation of antioxidant activity

The ferric reducing antioxidant power (FRAP) assay was employed to measure the total antioxidant capacity of the compounds, converting ferric tripyridyl triazine [Fe(III)-TPTZ] complex into a blue ferrous tripyridyl triazine [Fe(II)-TPTZ] complex at a low pH, measurable at 593 nm [31].

Reagents: (1) Buffer solution: 0.187 gm sodium acetate and 1.6 mL acetic acid dissolved in double-distilled water to make 100 mL. (2) TPTZ: 0.155 gm TPTZ was dissolved in 100 mL of 40-mM HCl. (3) $FeCl_3$ solution: 0.324 gm $FeCl_3$ was dissolved in 100 mL of distilled water. (4) Standard ascorbic acid: 0.176 gm of standard ascorbic acid was dissolved in 100 mL of distilled water.

Fe(II)-TPTZ(2,4,6-tripyridyl-*s*-triazine) reagent was prepared by mixing a 10.0 mL of TPTZ solution, 10 mL of $FeCl_36H_2O$ solution and 100 mL of acetate buffer at pH 3.6. A mixture of 200.0 mL of sample solution and 3 mL of Fe(II)-TPTZ reagent was incubated at 37 °C for 15 min. The absorbance of colour complex Fe(II)-TPTZ was measured at 593 nm using ascorbic acid as the standard. The results were expressed as ascorbic equivalent (mmol/100 gm compound). Ascorbic acid taken $= 1.99 \times 10^{-4}$ mm. Sample taken $= 0.04$ mg. The FRAP can be calculated using the following equation:

$$\text{FRAP value } (\text{mm A.A.}/100\,\text{gm sample}) = \frac{\Delta\text{OD593 nm of test sample} \times \text{standard (mm)} \times 105}{\Delta\text{OD593 nm of standard} \times \text{sample (mg)}}$$

Results and discussion

Characterization of compounds 4a–p

In the IR spectra, some significant stretching bands due to NH_2, C=O, C≡N and C–O–C are observed about 3470–3180, 1715–1635, 2205–2190 and 1210–1185 cm^{-1}, respectively. The ^1H NMR spectrum of compounds **4a–p** indicated the presence of one singlet in the range δ 6.44–6.60 ppm of a –CH proton of a C1H-pyr ring, and the disappearance of a singlet from δ 9.57–9.63 ppm of –CHO, clearly confirming the cyclization of the Knoevenagel intermediate. Moreover, multiplets in the range δ 7.46–8.29 ppm appeared for aromatic protons and all $-NH_2$ protons appear in the range 8.46–8.80 ppm. In the ^{13}C NMR spectral data of the title compounds **4a–p**, the most characteristic signal around δ 58.90–60.18 ppm (C1-pyr) indicated the formation of a pyrazolo[1,2-*b*]phthalazine ring. The signal at around δ 61.02–62.50 ppm (C2-pyr) is assigned to carbon attached to carbonitrile, and δ 81.90–82.55 ppm (C2-pyr) is assigned to carbon attached to an ester group. Also, δ 164.17–164.60 ppm is assigned to a carbonyl carbon of ester (O–C=O), while signals

around δ 116.05–160.20 ppm are attributed to all the aromatic carbons of compounds **4a–p**. The obtained elemental analysis values are in consonance with theoretical data. Mass spectra of title compounds showed expected molecular ion peak M^+ corresponding with proposed molecular mass.

Antimicrobial activity

Upon examination of bioactivity data of compounds **4a–p** (Table 1), it was noticed that almost all the compounds were equipotent or more potent compared to the standard drug ampicillin, and a few compounds were equipotent or more potent to

Table 1 In vitro antimicrobial activity of pyrazolo[1,2-*b*]phthalazine **4a–p**

Compound	Gram-positive bacteria			Gram-negative bacteria			Fungal species	
	Bs. MTCC 441	Ct. MTCC 449	Sp. MTCC 1936	Ec. MTCC 443	St. MTCC 98	Vc. MTCC 3906	Af. MTCC 3008	Ca. MTCC 227
Minimum inhibitory concentration (MIC) expressed in μg/mL								
4a	100	200	100	250	200	250	500	250
4b	500	250	250	500	500	500	200	1000
4c	250	200	250	100	200	250	>1000	500
4d	100	250	200	200	200	200	>1000	1000
4e	200	200	100	200	100	500	>1000	1000
4f	500	100	500	100	200	250	>1000	500
4g	200	100	100	100	50	200	500	100
4h	250	100	250	62.5	100	100	500	250
4i	100	250	100	200	200	250	250	1000
4j	250	250	250	200	250	250	1000	500
4k	250	200	500	62.5	250	250	>1000	250
4l	200	100	250	200	200	200	200	500
4m	100	200	200	100	200	100	1000	>1000
4n	250	250	250	200	250	250	1000	1000
4o	100	200	100	50	100	50	200	100
4p	250	100	500	250	25	125	200	250
A	250	250	100	100	100	100	–	–
B	50	100	50	25	25	25	–	–
C	100	50	10	10	10	10	–	–
D	50	50	50	50	50	50	–	–
E	–	–	–	–	–	–	100	100
F	–	–	–	–	–	–	100	500

Bs, *Bacillus subtilis*; Ct, *Clostridium tetani*; Sp, *Streptococcus pneumoniae*; Ec, *Escherichia coli*; St, *Salmonella typhi*; Vc, *Vibrio cholerae*; Af, *Aspergillus fumigatus*; Ca, *Candida albicans*; MTCC, Microbial Type Culture Collection; A, Ampicillin; B, Ciprofloxacine; C, Norfloxacine; D, Chloramphenicol; E, Nystatin; F, Griseofulvin. '–' represents 'not tested'

norfloxacin. Against Gram positive bacteria, *B. subtilis* compounds **4a**, **4d**, **4i**, **4m** and **4o** (MIC = 100 μg/mL) displayed efficacy, while compounds **4e**, **4g** and **4l** (MIC = 200 μg/mL) were far better than ampicillin (MIC = 250 μg/mL). For inhibiting *C. tetani*, compounds **4f**, **4g**, **4h**, **4l** and **4p** displayed efficacy (MIC = 100 μg/mL), while compounds **4a**, **4c**, **4e**, **4k**, **4m** and **4o** displayed (MIC = 200 μg/mL) activity much higher than that of ampicillin (MIC = 250 μg/mL). Against *S. pneumonia*, compounds **4a**, **4e**, **4g**, **4i** and **4o** (MIC = 100 μg/mL) were found to be equipotent to ampicillin while none of the compounds were found to be more potent than that of ampicillin. Also, compounds **4f**, **4g**, **4h**, **4l** and **4p** (MIC = 100 μg/mL) showed activity comparable to ciprofloxacine (MIC = 100 μg/mL) towards *C. tetani.* Compounds **4a**, **4d**, **4i** and **4o** (MIC = 100 μg/mL) were found to have activity comparable to norfloxacin (MIC = 100 μg/mL) towards *B. subtilis.*

Against Gram negative bacteria *E. coli,* compound **4o** (MIC = 50 μg/mL), **4h** and **4k** (MIC = 62.5 μg/mL) were found to be more potent whereas **4c**, **4g** and **4m** (MIC = 100 μg/mL) showed comparable activity to ampicillin (MIC = 100 μg/mL). Moreover, compound **4g** (MIC = 50 μg/mL) is found to possess pronounced activity against *S. typhi* compared to chlormphenicol (MIC = 50 μg/mL). Compounds **4e** and **4h** (MIC = 100 μg/mL) showed activity comparable to ampicillin (MIC = 100 μg/mL) towards *S. typhi.* Against *V. cholera*, compound **4o** (MIC = 50 μg/mL) was found to bear excellent activity upon comparison with ampicillin (MIC = 100 μg/mL), and was equipotent with chlormphenicol (MIC = 50 μg/mL).

Against fungal pathogen *C. albicans*, compounds **4g** and **4o** were found to possess excellent activity (MIC = 100 μg/mL), while compounds **4a**, **4h** and **4k** (MIC = 250 μg/mL) were found to be more potent than that of the standard drug griseofulvin (MIC = 500 μg/mL). None of the compounds were found to be active against fungal pathogen *A. fumigates*.

Antituberculosis activity

The encouraging results from the antimicrobial studies prompted us to proceed to the preliminary screening of the title compounds for their in vitro antituberculosis

Table 2 In vitro antituberculosis activity (% inhibition) of pyrazolo[1,2-*b*]phthalazine **4a–p** against *M. tuberculosis* H37Rv (at concentration 250 μg/mL)

Compound	% Inhibition	Compound	% Inhibition
4a	54	**4i**	33
4b	62	**4j**	48
4c	92	**4k**	95
4d	78	**4l**	82
4e	43	**4m**	37
4f	78	**4n**	63
4g	99	**4o**	96
4h	98	**4p**	82
Rifampicin	98		
Isoniazid	99		

activity against *M. tuberculosis* H37Rv bacteria (Table 2). Of the compounds screened for antituberculosis activity, compound **4g** (MIC = 25 mg/mL) was found to possess the highest potency against *M. tuberculosis* with 98 % inhibition as compared to rifampicin (MIC = 40 mg/mL). Compounds **4k** (MIC = 62.5 mg/mL) and **4o** (MIC = 50 mg/mL) exhibited inhibition of 95 % and 96 %, respectively. Also, compounds **4c** and **4h** (MIC = 100 mg/mL) displayed moderate inhibition of 92 and 91 %, respectively (Table 3).

It is interesting to note that substitutions at R_1, R_2 and R_3 positions make a wide impact on antituberculosis activity rather than substitutions at the R_4 position. Out of five active compounds, four compounds having $R_1 = CH_3$ and $R_2 = F$ effectively inhibited the growth of *M. tuberculosis* (i.e., **4c**, **4g**, **4k** and **4o** except **4h**). Also, at the R_3 position, the ester group has more impact than the cyanide group. So, there is a combination effect of CH_3, F and COOEt groups to improve the tuberculosis activity. Compound **4g** ($R_1 = CH_3$, $R_2 = F$, $R_3 = COOEt$) emerged out as the most potent member of the series and opens up a new door to optimize this series for a new class of antitubercular agents. From the antitubercular activity results, it is worth mentioning that the presence of lipophilic groups at the R_3 position improve the lipophilicity of the whole molecule. As a result, the molecule may be expected to more easily penetrate the bacterial cell line.

Cytotoxicity

The LC_{50} values obtained for the five compounds exhibiting the highest % inhibition are shown in Table 4. As can be seen, for the five compounds, there was no significant toxicity observed for compounds **4g**, **4h** and **4o** after a 24-h incubation. Among the compounds tested, compounds **4c** and **4k** showed greater toxicity.

Antioxidant activity

Examination of the data (Table 5) revealed that compounds **4g** and **4h** showed relatively high antioxidant power while compounds **4c**, **4d**, **4o** and **4p** were found to have better ferric reducing power. Compounds **4k** and **4l** displayed promising antioxidant potency. From the ferric reducing power results, it can be stated that compounds carrying an electron donating CH_3 group at the R_1 position and an ester

Table 3 In vitro antituberculosis activity of title compounds exhibiting higher % inhibition against *M. tuberculosis* H37Rv (MICs, mg/mL)

Compound	% Inhibition	MIC (mg/mL)
4c	92	100
4g	98	25
4h	91	100
4k	95	62.5
4o	96	50
Rifampicin	98	40
Isoniazid	99	0.20

Table 4 Cytotoxicity of title compounds exhibiting higher MICs against *M. tuberculosis* H37Rv (LC_{50}, mg/mL)

Compound	Concentration (C; mg/mL)	Log C	No. of nauplii taken	No. of nauplii dead	% Mortality	LC_{50} (mg/mL)
4c	5	0.699	10	1	10	18.91
	10	1.000		2	20	
	20	1.301		4	40	
	30	1.477		6	60	
	40	1.602		8	80	
	50	1.699		9	90	
4g	5	0.699	10	0	0	38.15
	10	1.000		1	10	
	20	1.301		2	20	
	30	1.477		4	40	
	40	1.602		5	50	
	50	1.699		7	70	
4h	5	0.699	10	1	10	25.22
	10	1.000		2	20	
	20	1.301		3	30	
	30	1.477		5	50	
	40	1.602		6	60	
	50	1.699		8	80	
4k	5	0.699	10	1	10	16.32
	10	1.000		3	30	
	20	1.301		5	50	
	30	1.477		7	70	
	40	1.602		8	80	
	50	1.699		9	90	
4o	5	0.699	10	0	0	33.12
	10	1.000		1	10	
	20	1.301		2	20	
	30	1.477		4	40	
	40	1.602		6	60	
	50	1.699		7	70	

group at the R_3 position exhibited excellent ferric reducing power, but when the electron-withdrawing group NO_2 entered the R_4 position, a decrease in the antioxidant activity occurred. In addition, it should be noted that the compounds with $R_1 = CH_3$ and $R_2 = F$ give better results than cyanide substitution ($R_2 = CN$). It may be due to the combination effect of $R_1 = CH_3$, $R_2 = F$ or CN and the ester group at the R_3 position.

In conclusion, we have demonstrated the use of NaOH as an efficient green reaction medium for the synthesis of 1*H*-pyrazolo[1,2-*b*]phthalazine-5,10-dione derivatives (i.e., **4a**–**p**) bearing 5-aryloxypyrazole for probing antimicrobial,

Table 5 In vitro antioxidant activity of compounds **4a–p** derivatives

Entry	OD (593 nm)	FRAP value[a]	Entry	OD (593 nm)	FRAP value[a]
4a	0.481	95.07	**4i**	0.185	36.56
4b	0.387	76.49	**4j**	0.082	16.20
4c	1.368	270.39	**4k**	0.775	153.18
4d	1.335	263.87	**4l**	0.690	136.38
4e	0.301	59.49	**4m**	0.208	41.11
4f	0.211	41.70	**4n**	0.170	33.60
4g	1.905	376.53	**4o**	0.953	188.36
4h	1.675	331.07	**4p**	0.980	193.70
A.A.	2.517	–	A.A.	2.517	–

Concentration of compounds used = 200 mg/mL

Concentration of standard (A.A.) = 176 mg/mL

A.A. = Ascorbic acid

[a] A.A. mm/100 gm sample

antituberculosis and antioxidant activity. Compounds **4e**, **4g**, **4h**, **4k** and **4o** exhibited excellent antimicrobial inhibition, while compounds **4c**, **4d**, **4g** and **4 h** showed the highest ferric reducing power. Compounds **4g** and **4o** emerged as the promising antimicrobial members with better antitubercular activity and lower toxicity. Consequently, such type of compounds represent a fertile matrix for further development of more biologically potent agents that deserve further investigation and derivatization in order to discover the scope and limitation of their biological activities.

Conclusion

A novel series of pyrazolo[1,2-*b*]phthalazine derivatives (**4a–p**) have been successfully synthesized and characterized. The antimicrobial activity results showed that compounds **4e**, **4g**, **4h**, **4k** and **4o** exhibited excellent antimicrobial activity compared with first line drugs. In vitro antituberculosis activity was evaluated against *M. tuberculosis* H37Rv and compounds **4g** and **4o** emerged as the promising antimicrobial members with better antituberculosis activity. Compounds **4c**, **4d**, **4g** and **4h** showed the highest antioxidant potency.

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