In vitro microbiological evaluation of 1,1’-(5,5’-(1,4-phenylene)bis(3-aryl-1H-pyrazole-5,1-(4H,5H)-diyl))diethanones, novel bisacetylated pyrazoles

Vijayakumar Kanagarajan1,2, Muthuvel Ramanathan Ezhilarasi2 and Mannathusamy Gopalakrishnan2*

Abstract
Novel 1,1’-(5,5’-(1,4-phenylene)bis(3-aryl-1H-pyrazole-5,1-(4H,5H)-diyl))diethanones 7-12 were tested for their antimicrobial activity by disc diffusion and twofold serial dilution method against the tested bacterial and fungal strains. Compounds 7 against Micrococcus luteus, 8 against β-Heamolytic streptococcus, M. luteus, Klebsiella pneumonia, Microsporum gypseum, 9 against Staphylococcus aureus, Shigella flexneri, Vibreo cholerae, Pseudomonas aeruginosa, Aspergillus flavus, Mucor indicus, 10 against Salmonella typhi, S. flexneri, M. gypseum, 11 against K. pneumonia, M. gypseum, 12 against K. pneumonia, and M. gypseum show superior zone of inhibitions and exhibited excellent antibacterial and antifungal activities at a MIC value of 6.25 μg/mL. Moreover, all the tested compounds 7-12 revealed promising antitubercular activity against Mycobacterium tuberculosis H37Rv and INH-resistant M. tuberculosis. Compounds 8 against M. tuberculosis and 11 against INH-resistant M. tuberculosis exhibited the percentage of reduction in RLU at 89 and 85%, respectively.

Keywords: bisacetylated pyrazoles, in situ acetylation, antibacterial activity, antifungal activity; antitubercular activity

1. Introduction
Mycobacterium tuberculosis (MTB) is a pathogenic bacterial species in the genus Mycobacterium and is the causative agent of most cases of tuberculosis. Tuberculosis is a common and often deadly infectious disease in humans [1,2]. Tuberculosis is the most common opportunistic disease in persons infected with human immunodeficiency virus [3]. The genome of MTB is rich in lipid-metabolizing and P450 enzymes. The cell envelope of MTB is unique and is associated with its pathogenicity [4]. Mycolic acids are the major constituents of the protective barrier of cell envelope of MTB and are essential for survival, virulence, and antibiotic resistance [5]. Inhibitors of mycolic acid biosynthesis, such as isoniazid (INH), ethambutol (EMB), and pyrazinamide (PZA), are still in the frontline of antitubercular drugs [6].

The discovery of the norfloxacin plays an important role in structure-activity relationships analysis of the fluoroquinolonic nucleus A-D, (Scheme 1) which led to the development of new derivatives with better solubility, higher antimicrobial activity, prolonged serum half-life, fewer adverse side effects, and both oral and parenteral routes of administration [7-9]. Naturally occurring bacterial DNA gyrase inhibitor such as novobiocin, a coumarin derivative E (Scheme 1), is known as antibacterial agents [10]. The coumarin drug inhibits ATPase activity of DNA gyrase by competing with ATP for binding to the subunit B of the enzyme. Owing to side effects, no pharmaceutically useful drug has been derived from the coumarins [11]. Although huge efforts have been dedicated to find a potent antibacterial agents that can overcome bacterial resistance, promising lead structures of DNA gyrase and topoisomerase IV enzyme inhibitors with novel mechanisms of action have not been found [12]. This reflects the inherent difficulties associated with the discovery and clinical testing of new candidates and the lack of significant pharmaceutical industry research in this area. Hence, the discovery and development of new drugs that effectively combat TB are accorded a great importance. In recent years, interest in pyrazoles has increased significantly because of their proven usefulness as intermediates in the preparation of new pharmaceuticals and agrochemicals.
[13-15]. Also, pyrazole derivatives F and G (Scheme 1) were identified as a new class of DNA gyrase and topoisomerase IV enzyme inhibitors [16]. Besides these, amides are well known for their therapeutic values since the amide group is an important pharmacophore. Antibiotics such as penicillins and cephalosporins have an amide group. The resistance toward available drugs is rapidly becoming a major worldwide problem. The necessity to design new compounds to overcome this resistance has become one of the most important areas of research today. Owing to our interest in synthesizing fascinating biologically active structurally diverse heterocycles [17-20], we recently reported the clean production of 1,1'-((5,5'-(1,4-phenylene)bis(3-aryl-1H-pyrazole-5,1-(4H,5H)-diyl))diethanones, a novel series of bis pyrazole derivatives using sodium acetate/acetic anhydride triggered by ultrasound irradiation [21], which accelerated the chemical reaction and mass transferred via the process of acoustic cavitation [22]. Extending the research in this area, we decided to investigate the antibacterial, antifungal, and antitubercular activities of the target compounds with the hope to develop some promising antimicrobial and antymycobacterial agents.

2. Experimental
2.1 Chemistry
Performing TLC assessed the reactions and the purity of the products. All the reported melting points are taken in open capillaries and were uncorrected. Sonication is performed on a Life Care-Fast Ultrasonic system (Life Care Equipments Pvt. Ltd., Mumbai, India) operating at a frequency of 45 kHz. The reaction flask is located in the maximum energy area in the bath and the addition or removal of water controlled the temperature of the water bath. IR spectra are recorded in KBr (pellet forms) on a Thermo Nicolet-Avatar-330 FT-IR spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, US) and note worthy absorption values (cm⁻¹) alone are listed. ¹H and ¹³C NMR spectra are recorded at 400 and 100 MHz, respectively, on Bruker AMX 400 NMR spectrometer (Bruker Biospin International, Ag, Aegeristrasse, Switzerland) using CDCl₃ as solvent. The ESI +ve MS spectra are recorded on a Varian Saturn 2200 MS spectrometer (Varian Inc., Palo Alto, USA). Satisfactory microanalyses are obtained on Carlo Erba 1106 CHN analyzer (Thermo Fisher Scientific Inc., Waltham, MA, US). By adopting the literature precedent, bis chalcones 1-6 [23] and 1,1'-((5,5'-(1,4-phenylene)bis(3-aryl-1H-pyrazole-5,1-(4H,5H)-diyl))diethanones 7-12 [21] are prepared.

2.2. Microbiology
All the clinically isolated bacterial strains namely Staphylococcus aureus, β-Heamolytic streptococcus, Micrococcus luteus, Bacillus subtilis, Salmonella typhii, Shigella flexneri, Vibrio cholerae, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, MTB H₃₇Rv, INH-resistant MTB and fungal strains namely Aspergillus flavus, Aspergillus niger, Mucor indicus, Rhizopus arrhizus, and Microsporum gypseum are obtained from the Faculty of Medicine, Annamalai University, Annamalainagar 608 002, Tamil Nadu, India.

2.3. In vitro antibacterial and antifungal activity by disc diffusion method
The in vitro activities of the compounds were tested in Sabourauds dextrose broth (SDB) (Hi-media, Mumbai) for fungi and nutrient broth (NB) (Hi-media, Mumbai) for bacteria by the disc diffusion method following the reported method [24]. The respective hydrochlorides of the test compounds 7-12 were dissolved in water to obtain 1 mg/mL stock solution and the different concentrations (100, 200, 500 ppm) were prepared from the stock solution. Seeded broth (broth-containing microbial spores) was prepared in NB from 24-h-old bacterial cultures on nutrient agar (Hi-media, Mumbai) at 37 ± 1°C while
fungal spores from 1 to 7-day-old Sabourauds agar (Hi-media, Mumbai) slant cultures were suspended in SDB. Sterile paper disc of 5-mm diameter was saturated with the three different concentrations and such discs were placed in each seeded agar plates. The petri plates were incubated in BOD incubator (Sigma Instruments, Chennai, India) at 37°C for bacteria and at 28°C for fungi. The zone of inhibition was recorded by visual observations after 24 h of inhibition for bacteria and after 72-96 h of inhibition for fungi. Moreover, the zone of inhibition was measured by excluding the diameter of the paper disc. Ciprofloxacin was used as standard for bacteria and fluconazole as standard for fungi under analogous conditions.

2.4. In vitro antibacterial and antifungal activity by twofold serial dilution method
MIC in μg/mL values was carried out by twofold serial dilution method [25]. The respective test compounds 7-12 were dissolved in dimethyl sulphoxide (DMSO) to obtain 1 mg/mL stock solution. Seeded broth (broth-containing microbial spores) was prepared in NB from 24-h-old bacterial cultures on nutrient agar (Hi-media, Mumbai) at 37 ± 1°C while fungal spores from 1 to 7-day-old Sabourauds agar (Hi-media, Mumbai) slant cultures were suspended in SDB. The colony forming units (cfu) of the seeded broth were determined by plating technique and adjusted in the range of 10^5-10^7 cfu/mL. The final inoculums size was 10^5 cfu/mL for antibacterial assay and 1.1-1.5 × 10^5 cfu/mL for antifungal assay. Testing was performed at pH 7.4 ± 0.2 for bacteria (NB) and at a pH 5.6 for fungi (SDB). Exactly 0.4 mL of the solution of test compound was added to 1.6 mL of seeded broth to form the first dilution. One milliliter of this was diluted with a further 1 mL of seeded broth to give the second dilution and so on till six such dilutions were obtained. A set of assay tubes containing only seeded broth was kept as control. The tubes were incubated in BOD incubators at 37 ± 1°C for bacteria and 28 ± 1°C for fungi. MICs were recorded by visual observations after 24 h (for bacteria) and 72-96 h (for fungi) of incubation. Ciprofloxacin was used as standard for bacteria studies and fluconazole was used as standard for fungal studies.

2.5. In vitro antitubercular activity by luciferase reporter phage assay method
The preliminary antitubercular activity screening was conducted against M. tuberculosis H37Rv, INH-resistant M. tuberculosis by luciferase reporter phage assay method [26] at two different concentrations (1.00 and 2.00 mg/mL). Fifty microliter bacterial suspension equivalent to MacFarlands No. 2 standard was added to 400 mL of G7H9 with and without the test compound. For each sample, two drug-free controls and two drug concentrations were prepared and this setup was incubated for 72 h at 37°C. After incubation, 50 mL of the high titer Luciferase reporter phage (PhAE129) and 40 mL of 0.1 M CaCl2 were added to all the vials and this setup was incubated at 37°C for 4 h. After incubation, 100 mL of the mixture was taken from each tube into a luminometer cuvette and equal amount of working D-Luciferin (0.3 mM in 0.05 M sodium citrate buffer, pH 4.5) solution was added. The RLU was measured after 10 s of integration in the Luminometer (Monolight 2010, Pegasus Scientific Inc., Rockville, USA). Duplicate readings were recorded for each sample and the mean was calculated. The percentage reduction in the RLU was calculated for each test sample and compared with control. The experiment was repeated when the mean RLU of the control was less than 1,000.

3. Results and discussion
3.1. Chemistry
Synthesis of 1,1’-(5,5’-(1,4-phenylene)bis(3-aryl-1H-pyrazole-5,1-(4H,5H)-diyl))diethanones 7-12 is carried out in excellent yields (Scheme 2 and Table 1) by the reaction of bis chalcones 1-6 with hydrazine hydrate catalyzed by anhydrous sodium acetate/acetac anhydride under ultrasonic irradiation method at 45°C within 10-20 min. It has been observed in the traditional classical method, the reaction mixture of bis chalcones 1-6 with hydrazine hydrate catalyzed by anhydrous sodium acetate in refluxing acetic anhydride for 5-8 h yield compounds 7-12 in moderate yields. However, when this reaction is performed under sonication method [27], the reaction takes place rapidly within 10-20 min with excellent yields (Table 1). In this study, acetic anhydride is the best solvent for the facile synthesis of bis pyrazoles, 7-12 in excellent yields without any solubility problem. In addition, in situ acetylation occurs in the course of the reaction because of solvent, acetic anhydride under the reaction conditions. The structures of the synthesized 1,1’-(5,5’-(1,4-phenylene)bis(3-aryl-1H-pyrazole-5,1-(4H,5H)-diyl))diethanones 7-12 are confirmed by FT-IR, MS, 1H NMR, and 13C NMR spectral studies and elemental analysis [21].

3.2. Antimicrobial activity of 1,1’-(5,5’-(1,4-phenylene)bis (3-aryl-1H-pyrazole-5,1-(4H, 5H)-diyl))diethanones by disc diffusion method 7-12
An array of biolabile 1,1’-(5,5’-(1,4-phenylene)bis(3-aryl-1H-pyrazole-5,1-(4H,5H)-diyl))diethanones 7-12 is tested for its antimicrobial activity by disc diffusion method against tested bacterial and fungal strains and the results are presented in Table 2. The use of 1,1’-(5,5’-(1,4-phenylene)bis(3-phenyl-1H-pyrazole-5,1-(4H,5H)-diyl))diethanone 7 shows good zone of inhibitions against M. luteus. Excellent zone of inhibitions is noted against S. aureus, B. H. streptococcos, M. luteus, B. subtilis, V. cholerae, E. coli, K. pneumonia, A. niger, and M. gypseum by compound 8.
which has electron withdrawing fluoro substituent at the para position of the phenyl ring. The usage of compound 9 which have electron withdrawing chloro substituent at the para position of the phenyl ring exhibits good zone of inhibitions against all the tested microorganisms except *S. typhii, E. coli*, and *M. gypseum*. Compound 10, which have electron withdrawing bromo substituent at the para position of the phenyl ring exhibits fine zone of inhibitions against all the tested bacterial strains except *K. pneumonia*. Excellent zone of inhibition is noticed by compound 10 against *M. gypseum*. The use of compound 11 which have electron donating methyl substituent at the para position of the phenyl ring exhibits superior zone of inhibitions against *S. flexneri, K. pneumonia, R. arrhizus*, and *M. gypseum*. Also, the use of compound 12 which have electron donating methoxy substituent at the para position of the phenyl ring exerts higher zone of inhibitions against *K. pneumonia, A. niger*, and *M. gypseum*.

### Table 1 Physical and analytical data of compounds 7-12

| Compounds | X             | Time Δ (h)/sonication (min) | Yield (%) Δ/sonication | m.p. (°C) | Elemental analysis (%) | m/z (M)\(^+\)molecular formula |
|-----------|---------------|----------------------------|------------------------|-----------|------------------------|--------------------------------|
| 7         | H             | 7/15                       | 65/95                  | 261       | 74.55 (74.65)          | 5.69 (5.82) 12.31 (12.44) 450 C\(_2\)H\(_2\)O\(_2\)N\(_2\) |
| 8         | F             | 7/15                       | 70/94                  | 233       | 69.02 (69.12)          | 4.77 (4.97) 11.41 (11.52) 486 C\(_2\)H\(_2\)F\(_2\)O\(_2\)N\(_2\) |
| 9         | Cl            | 8/20                       | 55/88                  | 260       | 64.52 (64.74)          | 4.52 (4.66) 10.66 (10.79) 518, 520 C\(_2\)H\(_2\)Cl\(_2\)O\(_2\)N\(_2\) |
| 10        | Br            | 7/15                       | 60/95                  | 262       | 55.13 (55.28)          | 3.82 (3.98) 9.11 (9.21) 606, 608 C\(_2\)H\(_2\)Br\(_2\)O\(_2\)N\(_2\) |
| 11        | CH\(_3\)       | 5/10                       | 65/98                  | 258       | 75.13 (75.29)          | 6.22 (6.32) 11.60 (11.71) 478 C\(_2\)H\(_2\)N\(_2\)O\(_2\) |
| 12        | OCH\(_3\)      | 5/10                       | 65/95                  | 202       | 70.43 (70.57)          | 5.86 (5.92) 10.85 (10.97) 510 C\(_2\)H\(_2\)N\(_2\)O\(_4\) |

3.3. Antimicrobial activity of 1,1’-(5,5’-(1,4-phenylene)bis(3-aryl-1H-pyrazole-5,1-(4H, 5H)-diyl))diethanones by twofold serial dilution method 7-12

In *vitro* antimicrobial results by the twofold serial dilution method (Table 3) of 1,1’-(5,5’-(1,4-phenylene)bis(3-aryl-1H-pyrazole-5,1-(4H, 5H)-diyl))diethanones 7-12 show that compound 7 exhibits good activities against *M. luteus* at a MIC value of 6.25 µg/mL. Admirable activities against *B. H. streptococcus, M. luteus, K. pneumonia*, and *M. gypseum* are displayed by compound 8 at a MIC value of 6.25 µg/mL, whereas it displays modest activities against *S. aureus* and *B. subtilis* at a MIC value of 12.5 µg/mL. The use of compound 9 displays higher activities against *S. aureus, S. flexneri, V. cholerae, P. aeruginosa, A. flavus*, and *M. indicus* at a MIC value of 6.25 µg/mL. Excellent antimicrobial activities are exhibited by compound 10 against *S. typhii, S. flexneri*, and *M. gypseum* at a MIC value of 6.25 µg/mL, whereas it exhibits superior activities against *V. cholerae, E. coli*, and *P. aeruginosa* at a MIC value of 12.5 µg/mL. The use of compound 11, which has electron donating methyl group at the para position of the phenyl ring, exhibits greater activities against *K. pneumonia and M. gypseum* at a MIC value of 6.25 µg/mL. Modest activities are displayed by compound 12 against *A. niger* at a MIC value of 12.5 µg/mL, whereas it exhibits strong activities against *K. pneumonia* and *M. gypseum* at a MIC value of 6.25 µg/mL.

3.4. Antitubercular activity of 1,1’-(5,5’-(1,4-phenylene)bis(3-aryl-1H-pyrazole-5,1-(4H, 5H)-diyl))diethanones by luciferase reporter phage assay method 7-12

In *vitro* antitubercular activity screening was evaluated against *M. tuberculosis H37Rv, INH-resistant M. tuberculosi*s by luciferase reporter phage assay method at two...
### Table 2 In vitro antibacterial and antifungal activities of compounds 7-12 by disc diffusion method

| Microorganisms                  | Compound 7 (ppm) | Compound 8 (ppm) | Compound 9 (ppm) | Compound 10 (ppm) | Compound 11 (ppm) | Compound 12 (ppm) |
|---------------------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
|                                 | 100 | 200 | 500 | 100 | 200 | 500 | 100 | 200 | 500 | 100 | 200 | 500 | 100 | 200 | 500 | 100 | 200 | 500 |
| Staphylococcus aureus           | ++  | ++  | +++ | -   | ++  | +++ | +++ | ++  | ++  | +++ | ++  | ++  | +++ | ++  | ++  | ++  | +++ | ++  | ++  | +++ |
| β-Heamolytic streptococcus      | ++  | ++  | +++ | ++  | +++ | +++ | +++ | ++  | ++  | +++ | ++  | ++  | +++ | ++  | ++  | ++  | +++ | ++  | ++  | +++ |
| Micrococcus luteus              | ++  | ++  | +++ | ++  | +++ | +++ | +++ | ++  | ++  | +++ | ++  | ++  | +++ | ++  | ++  | ++  | +++ | ++  | ++  | +++ |
| Bacillus subtilis               | ++  | ++  | +++ | ++  | +++ | +++ | +++ | -   | ++  | +++ | ++  | ++  | +++ | ++  | ++  | ++  | +++ | ++  | ++  | +++ |
| Salmonella typhii               | ++  | ++  | +++ | ++  | +++ | +++ | +++ | ++  | ++  | +++ | ++  | ++  | +++ | ++  | ++  | ++  | +++ | ++  | ++  | +++ |
| Shigella flexneri               | -   | ++  | ++  | ++  | ++  | ++  | ++  | ++  | ++  | ++  | ++  | ++  | ++  | ++  | ++  | ++  | ++  | ++  | ++  | ++  |
| Vibrio cholerae                 | -   | ++  | -   | +++ | +++ | +++ | +++ | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   |
| Escherichia coli                | ++  | ++  | +++ | ++  | +++ | +++ | +++ | ++  | ++  | +++ | ++  | ++  | +++ | ++  | ++  | ++  | +++ | ++  | ++  | +++ |
| Pseudomonas aeruginosa          | ++  | ++  | +++ | ++  | +++ | +++ | +++ | ++  | ++  | +++ | ++  | ++  | +++ | ++  | ++  | ++  | +++ | ++  | ++  | +++ |
| Klebsiella pneumonia            | ++  | ++  | +++ | ++  | +++ | +++ | +++ | ++  | ++  | +++ | ++  | ++  | +++ | ++  | ++  | ++  | +++ | ++  | ++  | +++ |
| Aspergillus flavus              | -   | ++  | ++  | ++  | ++  | ++  | ++  | ++  | ++  | ++  | ++  | ++  | ++  | ++  | ++  | ++  | ++  | ++  | ++  | ++  |
| Aspergillus niger               | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   |
| Mucor indicus                   | -   | ++  | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   |
| Rhizopus arrhizus               | -   | ++  | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   |
| Microsporum gypseum             | ++  | ++  | +++ | ++  | +++ | +++ | +++ | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   |

(-) = inactive, (+) = weakly active (12-16 mm), (+)(+) = moderately active (17-21 mm), (+)(+)(+) = strongly active (22-29 mm), (+)(+)(+)(+) = highly active (30-33 mm).

At 500 ppm concentration, standard antibacterial drug, ciprofloxacin exhibits 30 ± 0.5 mm zone of inhibition against all the test bacteria and standard antifungal drug, fluconazole exhibits 20 ± 0.5 mm zone of inhibition against all the test fungi.

### Table 3 In vitro antibacterial and antifungal activities of compounds 7-12 by twofold serial dilution method

| Microorganisms                  | Minimum inhibitory concentration (MIC) (μg/mL) |
|---------------------------------|-----------------------------------------------|
|                                 | 7    | 8    | 9    | 10   | 11   | 12   | Ciprofloxacin | Fluconazole |
| Staphylococcus aureus           | 50   | 12.5 | 6.25 | 25   | 200  | 100  | 25   | -           |
| β-Heamolytic streptococcus      | 50   | 6.25 | 25   | 25   | 100  | 200  | 25   | -           |
| Micrococcus luteus              | 6.25 | 6.25 | 25   | 25   | 200  | 200  | 12.5 | -           |
| Bacillus subtilis               | 50   | 12.5 | 25   | 25   | 200  | 200  | 12.5 | -           |
| Salmonella typhii               | 100  | 50   | 50   | 6.25 | 100  | 200  | 25   | -           |
| Shigella flexneri               | 200  | 100  | 6.25 | 6.25 | 25   | 100  | 12.5 | -           |
| Vibrio cholerae                 | 100  | 25   | 6.25 | 12.5 | 50   | 100  | 25   | -           |
| Escherichia coli                | 50   | 25   | 50   | 12.5 | 50   | 100  | 25   | -           |
| Pseudomonas aeruginosa          | 25   | 100  | 6.25 | 12.5 | 100  | 50   | 25   | -           |
| Klebsiella pneumonia            | 25   | 6.25 | 25   | 6.25 | 200  | 6.25 | 12.5 | -           |
| Aspergillus flavus              | 50   | 50   | 6.25 | 200  | 50   | 100  | -    | 12.5       |
| Aspergillus niger               | 200  | 25   | 25   | 100  | 50   | 12.5 | -    | 12.5       |
| Mucor indicus                   | 100  | 100  | 6.25 | 100  | 200  | 200  | -    | 25         |
| Rhizopus arrhizus               | 100  | 100  | 25   | 100  | 25   | 100  | -    | 25         |
| Microsporum gypseum             | 50   | 6.25 | 200  | 6.25 | 6.25 | 6.25 | -    | 12.5       |
different percentage inhibitions are summarized in Table 4. A compound is considered to possess antimycobacterial activity if 50% reduction in the relative light units (RLU) is observed when compared to the control using a luminometer. In vitro antimycobacterial activity results of 7-12 show that all the synthesized compounds exhibited good activity against the tested two M. tuberculosis bacterial strains, namely, M. tuberculosis H$_{37}$Rv and INH-resistant M. tuberculosis. It is observed from Table 4 that the activity of compounds get increased as the concentration of compound increases from 1.00 to 2.00 µg/mL. The percentage of reduction in RLU for the synthesized compounds is in the range of 74-88% against the tested bacterial strain M. tuberculosis H$_{37}$Rv and 73-85% against the tested bacterial strain INH-resistant M. tuberculosis. Among the synthesized compounds, compound 8 exhibited excellent antimycobacterial activity against M. tuberculosis H$_{37}$Rv and the percentage of reduction in RLU for 8 is 89%. Similarly, compound 11 exhibited excellent antimycobacterial activity against INH-resistant M. tuberculosis and the percentage of reduction in RLU for fluorine-substituted compound 8 is 85%. Also, fluorine substitution is commonly used in contemporary medicinal chemistry to improve metabolic stability, bioavailability, and protein-ligand interactions [28-30].

### 4. Conclusion

A clean, efficient, convenient, and economical synthesis of 1,1’-(5,5’-(1,4-phenylene)bis(3-aryl-1H-pyrazole-5,1-(4H,5H)-diyl))diethanones using ultrasound irradiation is described. The microbiological screening studies carried out to evaluate the antibacterial and antifungal potencies of the synthesized 1,1’-(5,5’-(1,4-phenylene)bis(3-aryl-1H-pyrazole-5,1-(4H,5H)-diyl))diethanones 7-12 were clearly known from Tables 2 and 3. In vitro antibacterial and antifungal activities profile of substituted aromatics (X = F, Cl, Br) are more active than non-substituted aromatic ring system (X = H) of novel target compounds exerted strong antibacterial and antifungal activity against all the tested bacterial strains. Among all the tested compounds, electron withdrawing-substituted compounds 8, 9, and 10 exerted moderate antimicrobial activity and the range of MIC values of 8-10 are 200-6.25 µg/mL. Among the synthesized compounds, compound 8 against M. tuberculosis and compound 11 against INH-resistant M. tuberculosis exhibited the percentage of reduction in RLU at 89 and 85%, respectively. Further development of this group of 1,1’-(5,5’-(1,4-phenylene)bis(3-aryl-1H-pyrazole-5,1-(4H,5H)-diyl))diethanones may lead to compounds with better pharmacological profile than standard antibacterial, antifungal, and antitubercular drugs that are under progress.

**Acknowledgements**

The authors wish to thank the NMR Research Centre, Indian Institute of Science, Bangalore, India, for recording spectra. V. Kanagarajan is grateful to the Council of Scientific and Industrial Research (CSIR), New Delhi, India, for providing financial support in the form of CSIR-Senior Research Fellowship (SRF) in Organic Chemistry. M. R. Ezhilarasi is thankful to Cavin Kare Research Centre, Chennai, for providing financial support in the form of Junior Research Fellowship.

**Author details**

1. Energetics Research Institute, Nanyang Technological University, 50, Nanyang Avenue, Singapore-639 798, Republic of Singapore. 2. Synthetic Organic Chemistry Laboratory, Department of Chemistry, Annamalai University, Annamalainagar 608 002, Tamil Nadu, India.

**Competing interests**

The authors declare that they have no competing interests.

**Received: 20 April 2011 Accepted: 20 September 2011 Published: 20 September 2011**

**References**

1. Kumar V, Abbas AK, Fausto N, Mitchell RN (2007) Robbins basic pathology. 8th edn. Saunders Elsevier, 8 pp 516–522.
2. Ryan KJ, Ray CG (2004) Shem’s medical microbiology, 4th edn. McGraw Hill.
3. Pereira M, Tripathy S, Inamdar V, Ramesh K, Bhavsar M, Date A, Iyyer R, Achammachary A, Mehdendale S, Rubad A (2005) Drug resistance pattern of Mycobacterium tuberculosis in seropositive and seronegative HIV-TB patients in Pune, India. Indian J Med Res 121:235–239.
4. Takayama K, Wang C, Besra GS (2005) Pathway to synthesis and processing of mycolic acids in Mycobacterium tuberculosis. Clin Microbiol Rev 18:81–101. doi:10.1128/CMR.18.1.81–101.2005.
5. Barry CE, Lee RE, Mduli K, Sampson AE, Schroeder BG, Sladen RA, Yuan Y (1998) Mycolic acids: structure, biosynthesis and physiological functions. Progress Lipid Res 37:143–179. doi:10.1016/S0163-7827(98)00008-3.
6. Zhang M, Yue J, Yang YP, Zhang HM, Lei JQ, An RL, Zhang XL, Wang XX (2005) Detection of mutations associated with isoniazid resistance in M. Tuberculosis. Compounds

| Compounds | M. Tuberculosis H$_{37}$Rv | INH resistant M. tuberculosis |
|-----------|--------------------------|-----------------------------|
|           | 1.00 (µg/mL) | 2.00 (µg/mL) | 1.00 (µg/mL) | 2.00 (µg/mL) |
| 7         | 74          | 78           | 73          | 77           |
| 8         | 85          | 89           | 79          | 82           |
| 9         | 82          | 85           | 79          | 82           |
| 10        | 79          | 81           | 78          | 80           |
| 11        | 81          | 83           | 82          | 85           |
| 12        | 82          | 84           | 80          | 84           |

Isoniazid
26. Sivakumar PM, Prabu Seenivasan S, Vanaja Kumar, Doble M (2007) Synthesis, antibacterial and antifungal activities of novel bisacetylated pyrazoles, Organic and Medicinal Chemistry Letters 2011 1:8.

27. Mason TJ, Lorimer JP (2002) Applied sonochemistry: the uses of power ultrasound in chemistry and processing. Wiley-VCH, Weinheim

28. Begue JP, Bonnet-Delpont D, (eds) (2008) Bioorganic and medicinal chemistry of fluorine. Wiley, Hoboken, NJ

29. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ (1997) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev 23:3-25.

30. Purser S, Moore PR, Swallow S, Gouverneur V (2008) Fluorine in medicinal chemistry. Chem Soc Rev 37:320-330. doi:10.1039/b610213c.