CHAPTER 2

Lower rim substituted 1, 3, 4-oxadiazole and 1, 3, 4-thiadiazole derivatives of calix[4]arene as antimicrobials, antimycobacterials and antioxidants

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Calix[4]arene based 1,3,4-oxadiazole and thiadiazole derivatives: Design, synthesis, and biological evaluation.
Abstract

In this chapter we describe the synthesis of some novel calixarene based heterocyclic compounds by coupling 5,11,17,23-tetra-tert-butyl-25,27-bis(chlorocarbonyl-methoxy)-26,28-dihydroxy calix[4]arene with 1,3,4-oxadiazole and 1,3,4-thiadiazole derivatives. All the newly synthesized compounds have been characterized by elemental analysis and various spectroscopic methods including FTIR, $^1$H NMR, $^{13}$C NMR and FAB-MS. These compounds were then subjected to in vitro antimicrobial screening against two Gram (+ve) bacteria (S. aureus, S. pyogenes), two Gram (-ve) bacteria (E. coli, P. aeruginosa) and two fungal strains (C. albicans, A. clavatus). They were also screened for their antioxidant activity and antitubercular activity against Mycobacterium tuberculosis H$_{37}$Rv.
2.1 Introduction

Calixarenes are oligomeric phenolic macrocycles that have excellent applications as molecular and metal receptors, biomolecule sensors and DNA binding agents [1-4]. But there have been very few reports that have focused on the intrinsic therapeutical properties of these macrocycles. There are a few hydrophilic derivates known that show interesting levels of activity against bacteria [5] fungi, cancerous cells and viruses [6,7], enveloped viruses [8], as well as against thrombosis [9] and fibrotic diseases [10]. The calixarene derivative ‘Macrocyclon’ [11] and more recently some parent species [12-14], were studied in the treatment of tuberculosis and other mycobacterioses. Functionalized calixarene mimics of vancomycin have also been studied as antimicrobial agents [15]. Some biological studies related to plasmid DNA binding, and cell transfection have notably been reported by Ungaro and co-workers [16-18]. Regnouf and coworkers, focused on the development of calixarene platforms designed molecular drug dispensers offering penicillin and quinolone moieties at the lower rim [19-22]. Ionic calixarene derivatives exhibit intrinsic antimicrobial activity [23-26], while p-guanidino ethyl calixarene and parent phenol derivatives exhibited antibacterial activities [27]. Hydroxycinnamic acid based derivatives also have been reported as radical scavenging agents having antioxidant activity [28].

A common feature of most of these biological active calixarenes is the presence of a heterocycle like the guanidine ring, quinolones, etc. These heterocyclic compounds play very major role in medicinal chemistry. Synthetic heterocyclic drugs are used as hypnotics, anticonvulsants, antiseptics, antineoplastics, antiviral, antihistaminics, anti-tumor etc. Majority of the large number of drugs being introduced in pharmacopeias every year are heterocyclic compounds.

1,3,4-oxadiazole, its sulphur analogue i.e thiadiazole and their derivatives constitute an important family of heterocyclic compounds. Due to their remarkable unique properties, they exhibit diverse biological activities such as antimicrobial [29, 30], anti-HIV [31], antitubercular [32, 33], antioxidant [34] and antimalarial [35].
2.1.1 Importance of oxadiazole and thiadiazole

1,3,4-oxadiazole is a heterocyclic compound containing an oxygen atom and two nitrogen atoms in a five-membered ring. It has four isomers but 1,3,4-oxadiazoles are better known and more widely studied by researchers because of their many important chemical and biological properties. (Figure 1)

![Figure 1. Isomers of oxadiazole](image)

1,3,4-Oxadiazole 1,2,3-Oxadiazole 1,2,4-Oxadiazole 1,2,5-Oxadiazole

[I] [II] [III] [IV]

1,3,4-thiadiazole is a heterocyclic molecule with one S atom and two N atoms. There are four isomers of thiadiazole 1,3,4-thiadiazole [I], 1,2,3-thiadiazole [II], 1,2,4-thiadiazole [III] and 1,2,5-thiadiazole [IV]. (Figure 2)

![Figure 2. Isomers of thiadiazole.](image)

1,3,4-thiadiazole, 1,2,3-thiadiazole, 1,2,4-thiadiazole, 1,2,5-thiadiazole

[I] [II] [III] [IV]

2.1.2 Antimicrobial activity of oxa/thiadiazole

1,3,4 oxadiazoles substituted with benzothiophene (b) [36], phenylamine [37], naphthyl derivatives [38] and 2-fluoro-4-methoxy moiety (a) [39] have been reported as excellent antibacterials and antifungals. (Figure 3)
During recent years, intense investigations on 1,3,4-oxadiazole compounds have revealed their remarkable properties as antituberculosis agents. Oxadiazoles having pyrazol-3-one (a, b) derivatives, have been studied as antitubercular agents and were found to have MIC, as low as 0.78 and 3.12 μg/mL respectively and free from any cytotoxicity (>62.5 μg/mL) [40,41]. Oxadiazoles with cyclic amines (c), (Figure 4) as substituents showed activities comparable to isoniazid and ofloxacin [42].

Figure 3. Some substituted 1,3,4-oxadiazole

2.1.3 Antimycobacterial activity

Thiadiazole having substituents like 1,2,4-triazole [43,44], benzotriazole [45], benzimidazole (b) [46], thiazole [47] and quinolines (a) [48] have been reported for their antimicrobial activity. (Figure 5)
2.1.4 Antimycobacterial activity of thiadiazole

Synthesis and *in-vitro* antimycobacterial activity of thiadiazoles having substituted thiophenes (a) [49], substituted imidazoles (b) [50], thiourea (c) [51] has been reported. A series of 2-sulfonamido/trifluoromethyl-6-(40-substituted aryl/heteroaryl) imidazo[2,1-b]-1,3,4-thiadiazole derivatives (D) have been synthesized and evaluated for antituberculc activity [52]. (Figure 6)

![Figure 5. Some substituted 1,3,4-thiadiazole](image)

*Figure 5. Some substituted 1,3,4-thiadiazole*

![Figure 6. Antimycobacterial activity of some 1,3,4-thiadiazole compounds](image)

*Figure 6. Antimycobacterial activity of some 1,3,4-thiadiazole compounds*

The therapeutic importance of these rings prompted us to develop molecules having calixarene platforms with these rings as substituents. The substituents arranged in a pharmacophore pattern would enhance the therapeutic effect of the calixarene moiety and hence enable to display higher pharmacological activity.

Hence in the present investigation, the synthesis and characterization of novel calix[4]arene derivatives incorporating two oxadiazole and thiadiazole subunits at the lower rim was carried out. The lipophilicity of the calixarene basket in cooperation with the lower rim subunits was anticipated to display excellent antibacterial activity and antifungal activity. We also have evaluated their antioxidant activity and antituberculc activity against *H. pylori* bacteria.
2.2 Experimental

2.2.1 Chemical and Reagents

All other chemicals and solvents used were of analytical grade of BDH, E-Merck or Qualigens and were used without further purification.

2.2.2 Apparatus

The melting points (°C, uncorrected) were taken using Veego Mel-Temp apparatus. The FT-IR spectra were recorded on Bruker Tensor 27 FT-IR spectrometer with KBr pellets. The FAB-MS were recorded on a Jeol /SX/ 102/Da-600 mass spectrometer data system using Argon/Xenon as the accelerating gas. m-Nitro benzyl alcohol (NBA) was used as a matrix with the peak at m/z 136, 137, 154, 289 and 307. GmbH Vario Micro cube elementar analyzer was used for elemental analysis. $^1$H NMR and $^{13}$C NMR spectra were recorded at 400 MHz & 500 MHz and 125 MHz respectively on a Bruker Avance II 400 spectrophotometer in DMSO-d₆ with tetra methyl silane (TMS) as an internal standard.

2.3 Synthesis and characterization

Scheme 1. Synthesis of hydrazides derivatives of nicotinic acid, benzoic acid and cis-cinnamic acid
Scheme 2. Synthesis of isoniazid, nicotinic, benzoic and cis-cinnamic hydrazide derivatives
Scheme 3. Synthesis of calix[4]arene-based 1,3,4-oxadiazole and 1,3,4-thiadiazole derivatives of isoniazid, nicotinic acid, benzoic acid and cis-cinnamic acid.

2.3.1 Synthesis of nicotinic acid, benzoic acid and cis-cinnamic acid hydrazide

The acid hydrazides of Nicotinic acid, benzoic acid and cis-cinnamic acid were synthesized by an earlier reported procedure [53, 54].

2.3.2 Synthesis of 1,3,4-oxadiazole, 1,3,4-thiadiazole derivatives of isoniazid, nicotinic acid hydrazide, benzoic acid hydrazide and cis-cinnamic acid hydrazide.

These compounds A, B, D, E, F, G, H and I were prepared according to a reported method [55-57].
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General Procedure for synthesis of compounds A, D, F and H:

A mixture of acid hydrazide (0.005 mol), KOH (0.005 mol) and carbon disulfide (5 mL) in ethanol (50 mL) was refluxed on a steam bath for 12 h. The solution was then concentrated, cooled and acidified with 1N HCl. The solid mass that separated out was filtered, washed with ethanol; dried solid was purified by crystallization from absolute alcohol to afford the desired compounds (A, D, F & H). (Scheme 2)

2.3.2.1 5-(pyridin-4-yl)-1,3,4-oxadiazole-2-thiol (A):
Yield: 74%, mp 166 °C; Anal. Calcd for C7H5N3OS (179.20): C, 46.92; H, 2.81; N, 23.45; O, 8.93; S, 17.89. Found: C, 46.95; H, 2.83; N, 23.48; O, 8.90; S, 17.91 %. FT-IR (KBr, \( \nu \) cm\(^{-1} \)): 1638 (C=O), 1521 (C=C aromatic), 1430 (C–O–C oxadiazole), 1164 (SH); \(^1\)H NMR (400 MHz, DMSO-d\(_6\)): \( \delta \) 7.94, 8.74 (m, 4H, Py), 13.03 (s, 1H, SH); MS: m/z 179 (M+).

2.3.2.2 5-(pyridin-3-yl)-1,3,4-oxadiazole-2-thiol (D):
Yield 74%, mp 231 °C; Anal. Calcd for C7H5N3OS (179.20): C, 46.92; H, 2.81; N, 23.45; O, 8.93; S, 17.89. Found: C, 46.93; H, 2.83; N, 23.46; O, 8.91; S, 17.90 %. FT-IR (KBr, \( \nu \) cm\(^{-1} \)): 1640 (C=N), 1510 (C=C aromatic), 1420 (C–O–C oxadiazole), 1167 (SH); \(^1\)H NMR (400 MHz, DMSO-d\(_6\)): \( \delta \) 7.90, 8.72 (m, 4H, Py), 13.02 (s, 1H, SH); MS: m/z 179 (M+).

2.3.2.3 5-(phenyl)-1,3,4-oxadiazole-2-thiol (F):
Yield: 74%, mp 219 °C; Anal. Calcd for C8H6N2OS (178.21): C, 53.92; H, 3.39; N, 15.72; O, 8.98; S, 17.99. Found: C, 53.94; H, 3.41; N, 15.75; O, 8.97; S, 17.89 %. FT-IR (KBr, \( \nu \) cm\(^{-1} \)): 2940 (C-H), 1510 (C=C aromatic), 1425 (C–O–C oxadiazole), 1160 (SH); \(^1\)H NMR (400 MHz, DMSO-d\(_6\)): \( \delta \) 7.94, 8.74 (m, 4H, Phenyl), 12.92 (s, 1H, SH); MS: m/z 178 (M+).

2.3.2.4 Cis-5-(styryl)-1,3,4-oxadiazole-2-thiol (H):
Yield: 74%, mp 167 °C; Anal. Calcd for C10H8N2OS (204.04): C, 58.80; H, 3.95; N, 13.72; O, 7.83; S, 15.70. Found: C, 58.81; H, 3.93; N, 13.70; O, 7.81; S, 15.72 %. FT-IR (KBr, \( \nu \) cm\(^{-1} \)): 2980 (C–H), 1515 (C=C aromatic), 1430 (C–O–C oxadiazole), 1161 (SH); \(^1\)H NMR (400 MHz, DMSO-d\(_6\)): \( \delta \) 7.94, 8.74 (m, 4H, Phenyl), 6.95-6.95 (dd, 2H, Styril), 13.00 (s, 1H, SH); MS: m/z 204 (M+).

General procedure for synthesis of B, E, G, and I

The acid hydrazide (0.01 mol) was dissolved in a minimum amount of 1N HCl and ammonium thiocyanate (0.02 mol) was added afterwards. The reaction mixture was heated under reflux for 8-10 hr. After cooling, the product was filtered, washed with water and recrystallized from absolute alcohol to give the assorted thiosemicarbazides.
The thiosemicarbazides (0.01 mol) was dissolved in 4 mL of conc. sulphuric acid. Then, the solution was kept at room temperature for 2 h, stirred occasionally and poured over crushed ice. The resulting solid was kept in ammoniacal water for 2 h. Then solid product was filtered, washed with water and dried. The crude was purified by crystallization from absolute alcohol to afford the desired compounds (B, E, G, & I) (Scheme 2)

23.2.5 2-Amino-5-(4'-pyridyl)-1,3,4-thiadiazole (B):
Yield 70%, mp 240 °C; Anal. Calcd. for C_{7}H_{6}N_{4}S (178.21): C, 47.18; H, 3.39; N, 31.44; S, 17.99. Found: C, 47.15; H, 3.40; N, 31.42; S, 17.97 %. FT-IR (KBr, v cm^{-1}): 3340 (N-H str.), 1621-1433 (ON), 660 (C-S-C); ^1H NMR (400 MHz, DMSO-d6): δ 8.50 (d, 2H, Ar-H, pyridine), 6.51 (s, 2H, NH2); MS: m/z 178 (M+).

23.2.6 2-Amino-5-(3'-pyridyl)-1,3,4-thiadiazole (E):
Yield 70%, mp 235 °C; Anal. Calcd. for C_{7}H_{5}N_{4}S (178.21): C, 47.18; H, 3.39; N, 31.44; S, 17.99. Found: C, 47.16; H, 3.41; N, 31.41; S, 17.98 %. FT-IR (KBr, v cm^{-1}): 3344 (N-H str.), 1625-1430 (ON), 664 (C-S-C); ^1H NMR (400 MHz, DMSO-d6): δ 8.48 (d, 2H, Ar-H, pyridine), 6.45 (s, 2H, NH2); MS: m/z 178 (M+).

23.2.7 2-Amino-5-(phenyl)-1,3,4-thiadiazole (G):
Yield 70%, mp 223 °C; Anal. Calcd. for C_{8}H_{6}N_{3}S (177.04): C, 54.22; H, 3.98; N, 23.71; S, 18.09. Found: C, 54.20; H, 3.97; N, 23.70; S, 18.11 %. FT-IR (KBr, v cm^{-1}): 3342 (N-H str.), 1620-1438 (C=N), 656 (C-S-C); ^1H NMR (400 MHz, DMSO-d6): δ 8.46 (d, 2H, Ar-H), 6.40 (s, 2H, NH2); MS: m/z 177 (M+).

23.2.8 Cis-2-Amino-5-(styryl)-1,3,4-thiadiazole (I):
Yield 70%, mp 165 °C; Anal. Calcd. for C_{10}H_{9}N_{3}S (203.26): C, 59.09; H, 4.46; N, 20.67; S, 15.78. Found: C, 59.11; H, 4.47; N, 20.70; S, 15.77 %. FT-IR (KBr, v cm^{-1}): 3342 (N-H str.), 2980 (C-H str.), 656 (C-S-C); ^1H NMR (400 MHz, DMSO-d6): δ 8.46 (d, 2H, Ar-H), 6.40 (s, 3H, NH2); MS: m/z 203 (M+).

23.2.9 2-Amino-5-(pyridin-4-yl)-1,3,4-oxadiazole (C):
The mixture of isoniazid (1.37 g, 0.01 mol) in minimum amount of methanol (20 mL) and cyanogen bromide (1.059 g, 0.01 mol) was stirred and refluxed at 55-56 °C for 2 h. The resulting solution was cooled & neutralized with a sodium bicarbonate solution. The solid thus precipitated was washed, dried and recrystallized from ethanol.

Yield 77%; mp 240 °C (dec); Anal. Calcd. for C_{8}H_{10}N_{4}O_{4} (178.19): C, 53.92; H, 5.66; N, 31.44; O, 8.98. Found: C, 53.90; H, 5.63; N, 31.44; O, 8.97 %. FT-IR (KBr, v cm^{-1}): 3340 (str...
NH), 1621-14330 (C=N), 1210 (C-O-C), \( ^1H\) NMR (DMSO-\(d_6\)) \( \delta \) 8.50 (m, 4H pyridine), 6.51 (s, 2H, NH\(_2\)); \( ^{13}C\) NMR (DMSO-\(d_6\)) \( \delta \) 169.2-153 (C-2 and C-5 of oxadiazole): 149.8, 124.2, 147 (C-4 of pyridine); MS: m/z 178 (M+).

2.3.3 Synthesis of compounds 1, 2, 3 and 4

The compounds 1, 2, 3 and 4 were prepared as reported in an earlier paper [58]. (Scheme 3)

2.3.4 General method for the synthesis of (5a-5i)

The novel lower rim substituted calixarene derivatives, 5a-5i were synthesized as given below.

The compound 4 5,11,17,23-tetra-tert-butyl-25,27-bis(chlorocarbonyl-methoxy)-26,28-dihydroxycalix[4]arene (2.07 mmol), obtained in the previous step was dissolved in dry THF (100 mL). The addition of pyridine (1 mL, 12.6 mmol) was done dropwise and the solution of appropriate 1,3,4-oxadiazole,1,3,4-thiadiazole (A - I) (7.30 mmol) in THF (25 mL) was added dropwise in about 1 h with continuous stirring at room temperature (37 °C). The reaction mixture was then stirred and refluxed for 5 h, after which most of the solvent was distilled off under vacuum. The residue was dissolved in 200 mL water and was neutralized with 0.1 M HCl. The solid material was then filtered and washed with 2 N HCl, NaHCO\(_3\) and distilled water sequentially. The crude product was purified by crystallization from ethanol-THF. The compounds (5a-5i) were synthesized in an analogous manner and were characterized as shown below.

2.3.4.1 5,11,17,23-tetra-tert-butyl-25,27-bis(5-(pyridin-4-yl)-1,3,4-oxadiazole-2-thiacarbonylmethoxy)-26,28-dihydroxycalix[4]arene (5a):

Yield 84%; mp > 250 °C; Anal.Calcd for C\(_{62}\)H\(_{66}\)N\(_6\) O\(_8\)S\(_2\) (1087.35): C, 68.48; H, 6.12; N,7.73; O, 11.77; S, 5.90. Found: C, 68.45; H, 6.14; N, 7.73; O, 11.76; S, 5.89 %; FT-IR (KBr, \( \nu \) cm\(^{-1}\)), 3400 (-OH), 3080 (-Ar CH), 1710 (-C=O), 1731, 1025 (C-O-C linkage), 1598, 1434, 1366 (oxadiazole ring str); \( ^1H\) NMR (400 MHz, DMSO-\(d_6\)) \( \delta \) 0.97 (s, 18H, tert-butyl), 1.20 (s, 18H, tert-butyl), 3.86 (d, 4H, J=12.8 Hz, Ar-CH\(_2\)-Ar), 4.25 (d, 4H, J=12.8 Hz, Ar-CH\(_2\)-Ar), 4.17 (s, 4H, OCH\(_3\)), 6.69-7.14 (m, 8H, ArH), 8.03 (s, 2H, OH), 7.25-8.50 (s, 8H, PyH); \( ^{13}C\) NMR (125 MHz, DMSO-\(d_6\)) \( \delta \) 31.2, 33.2 34.5, 32.2 (Me\(_3\)C of tert-butyl), 32.2 (Ar-CH\(_2\)-Ar), 122.4 , 125,
126.4, 133.2, 141.7, 147.3, 150.2, (-CH of Ar), 167.2 (SCO), 64.3 (OCH2), 120.3, 137.4, 150.2 (C1-C5 of pyridine ring), 164.5-167.5 (C6-C7 of oxadiazole ring); FAB-MS (m/z) 1088 (M+1).

23.4.2 5,11,17,23-tetra-tert-butyl-25,27-bis(5-(pyridin-4-yl)-1,3,4-thiadiazole-2-amidemethylen epoxy)-26,28-dihydroxycalix[4]arene (5b) :

Yield 82%; mp > 250 °C; Anal. Calcd for C62H68N8O8S2 (1085.38): C, 68.61; H, 6.27; N, 10.31; O, 8.82; S, 5.91. Found: C, 68.59; H, 6.26; N, 10.28; O, 8.80; S, 5.89%; FT-IR (KBr, v cm⁻¹): 3412 (-OH), 2989 (-Ar-CH), 1670-1656 (NHCO), 696 (C-S-C linkage), 1240 (N-N=C thiadiazole ring str); ¹H NMR (400 MHz, DMSO-d6), δ 0.94 (s, 18H, tert-butyl), 1.10 (s, 18H, tert-butyl), 3.80 (d, 4H, J=12.8 Hz, Ar-CH2-Ar), 4.10 (d, 4H, J=12.8 Hz, Ar-CH2-Ar), 4.39 (s, 4H, CH2O), 6.70-7.15 (m, 8H, ArH), 8.05 (s, 2H, OH), 7.35 (s, 8H, pyH), 10.20 (d, 2H, NH), ¹³C NMR (125 MHz, DMSO-d6) δ 31.4, 32.9, 34.2, 33.4 (Me3C), 32.2 (Ar-CH2-Ar), 124.4, 125.9, 127.4, 133.2, 143.7, 147.3, 149.1, 151 (CH of Ar), 168.5 (NHCO of amide), 63.3 (OCH2), 121.3, 137.2, 149.4 (C1-C5 of pyridine ring), 163.5-166.1 (C6-C7 of thiadiazole ring); FAB-MS (m/z) 1086 (M+1).

23.4.3 5,11,17,23-tetra-tert-butyl-25,27-bis(5-(pyridin-4-yl)-1,3,4-oxadiazole-2-amidemethylen epoxy)-26,28-dihydroxycalix[4]arene (5c) :

Yield 85%; mp > 250 °C; Anal. Calcd for C62H68N8O8 (1053.26): C, 70.70; H, 6.51; N, 10.64; O, 12.14. Found: C, 70.69; H, 6.48; N, 10.64; O, 12.15%; FT-IR (KBr, v cm⁻¹): 3410 (-OH), 2990 (-Ar-CH), 1670-1654 (NHCO), 1210, 1025 (C-O-C linkage), 1598, 1434, 1366 (oxadiazole ring str); ¹H NMR (400 MHz, DMSO-d6) δ 0.94 (s, 18H, tert-butyl), 1.15 (s, 18H, tert-butyl), 3.85 (d, 4H, J=12.8 Hz, Ar-CH2-Ar), 4.15 (d, 4H, J=12.8 Hz, Ar-CH2-Ar), 5.18 (s, 4H, CH2O), 6.70-7.10 (m, 8H, ArH), 8.20 (s, 2H, OH), 7.35 (s, 8H, pyH), 10.25 (d, 2H, NH), ¹³C NMR (125 MHz, DMSO-d6) δ 31.43, 32.2, 34.2, 34.30 (Me3C), 32.2 (Ar-CH2-Ar), 124.4, 125.9, 122.4, 133.2, 143.7, 147.3, 149.1, 151 (CH of Ar), 168.5 (NHCO of amide), 63.3 (OCH2), 121.3, 137.2, 149.4 (C1-C5 of pyridine ring), 163.5-166.1 (C6-C7 of an oxadiazole ring); FAB-MS (m/z) 1054 (M+1).

23.4.4 5,11,17,23-tetra-tert-butyl-25,27-bis(5-(pyridin-3-yl)-1,3,4-oxadiazole-2-thiacarbonyl methoxy)-26,28-dihydroxycalix[4]arene (5d) :

Yield 81%; mp > 250 °C; Anal. Calcd for C62H68N6O8S2 (1087.35): C, 68.48; H, 6.12; N, 7.73; O, 11.77; S, 5.90. Found: C, 68.47; H, 6.14; N, 7.75; O, 11.76; S, 5.87%; FT-IR (KBr, v cm⁻¹): 3410 (-OH), 3030 (-Ar-CH), 1710 (-C=O), 1731, 1015 (C-O-C linkage), 1600, 1424, 1356 (oxadiazole ring str); ¹H NMR (500 MHz, DMSO-d6) δ 0.94 (s, 18H, tert-butyl), 1.10 (s, 18H, tert-butyl), 3.76 (d, 4H, J=12.8 Hz, Ar-CH2-Ar), 4.35 (d, 4H, J=12.8 Hz, Ar-CH2-Ar), 4.14 (s, 4H, OCH2), 6.59-7.10 (m, 8H, ArH), 8.10 (s, 2H, OH), 7.20-9.10 (s, 8H, PyH), ¹³C NMR (125
MHz, DMSO-d$_6$  $\delta$ 31.2, 33.20, 34.5, 32.2 (Me$_3$C of tert-butyl), 32.2 (Ar-CH$_2$-Ar), 122.4, 125, 126.4 133.2 141.7 147.3. 150.2, (CH of Ar), 167.5 (SCO), 64.3 (OCH$_2$), 120.3-150.2 (C1-C5 of pyridine ring), 165.5-167 (C6-C7 of oxadiazole ring); FAB-MS (m/z) 1088 (M+1).

### 23.4.5 5,11,17,23-tetra-tert-butyl-25,27-bis(5-(pyridin-3-yl)-1,3,4-thiadiazole-2-amidemethylenoxy)-26,28-dihydroxycalix[4]arene (5e):

Yield 80%; mp > 250 °C; Anal. Calcd for C$_{62}$H$_{68}$N$_8$O$_6$S$_2$ (1085.38): C, 68.61; H, 6.31; N, 10.32; O, 5.88 %. FT-IR (KBr, $\nu$ cm$^{-1}$): 3420 (-OH), 3015 (-Ar-CH), 1670-1650 (NHCO), 685 (C-S-C linkage), 1230 (N-N=C thiadiazole ring str); $^1$H NMR (500 MHz, DMSO-d$_6$) $\delta$ 0.92 (s, 18H, tert-butyl), 1.15 (s, 18H, tert-butyl), 3.82 (d, 4H, $J$=12.8 Hz, Ar-CH$_2$-Ar), 4.10 (d, 4H, $J$=13.2 Hz, Ar-CH$_2$-Ar), 4.39 (s, 4H, CH$_2$O), 6.70-7.15 (m, 8H, ArH), 8.05 (s, 2H, OH), 7.35-9.15 (s, 8H, pyH), 10.10 (d, 2H, NH), $^{13}$C NMR (125 MHz, DMSO-d$_6$) $\delta$ 31.4, 32.9, 34.2, 33.4 (Me$_3$C), 32.2 (Ar-CH$_2$-Ar), 124.4, 125.9, 127.4, 133.2, 143.7, 147.3, 149.1, 151 (CH of Ar), 168.5 (NHCO of amide), 66.50 (OCH$_2$), 121.3-167.4 (C1-C5 of pyridine ring), 153.5-164.1 (C6-C7 of thiadiazole ring); FAB-MS (m/z) 1086 (M+1).

### 23.4.6 5,11,17,23-tetra-tert-butyl-25,27-bis(5-(phenyl)-1,3,4-oxadiazole-2-thiacarbonylmethoxy)-26,28-dihydroxycalix[4]arene (5f):

Yield 81%; mp > 250 °C; Anal. Calcd for C$_{62}$H$_{66}$N$_6$O$_6$S$_2$ (1085.38): C, 70.82; H, 6.31; N, 5.16; O, 11.79; S, 5.91. Found: C, 70.80; H, 6.28; N, 11.89; S, 5.92 %. FT-IR (KBr, $\nu$ cm$^{-1}$), 3440 (-OH), 3010 (-Ar-CH), 1715 (-C=O), 1735, 1005 (C-O-C linkage), 1610, 1414, 1346 (oxadiazole ring str); $^1$H NMR (500 MHz, DMSO-d$_6$) $\delta$ 0.95 (s, 18H, tert-butyl), 0.99 (s, 18H, tert-butyl), 3.96 (d, 4H, $J$=12.7 Hz, Ar-CH$_2$-Ar), 4.35 (d, 4H, $J$=13.1 Hz, Ar-CH$_2$-Ar), 4.4 (s, 4H, OCH$_2$), 6.59-7.24 (m, 8H, ArH), 8.10 (s, 2H, OH), 7.20-8.20 (s, 8H, PyH), $^{13}$C NMR (125 MHz, DMSO-d$_6$) $\delta$ 31.3, 33.2, 34.5, 32.3 (Me$_3$C of tert-butyl), 32.4 (Ar-CH$_2$-Ar), 122.4, 125, 126.4 133.2 141.7 147.3. 150.2, (CH of Ar), 164.5 (SCO), 64.4 (OCH$_2$), 121.3-153.2 (C1-C5 of the pyridine ring), 162.5-168 (C6-C7 of oxadiazole ring); FAB-MS (m/z) 1086 (M+1).

### 23.4.7 5,11,17,23-tetra-tert-butyl-25,27-bis(5-(phenyl)-1,3,4-thiadiazole-2-amidemethylenoxy)-26,28-dihydroxycalix[4]arene (5g):

Yield 80%; mp > 250 °C; Anal. Calcd for C$_{62}$H$_{68}$N$_8$O$_6$S$_2$ (1083.41): C, 70.95; H, 6.51; N, 7.76; O, 8.86; S, 5.92. Found: C, 70.93; H, 6.54; N, 7.74; O, 8.88; S, 5.94 %. FT-IR (KBr, $\nu$ cm$^{-1}$): 3400 (-OH), 3025 (-Ar-CH), 1650-1666 (NHCO), 687 (C-S-C linkage), 1243 (N-N=C thiadiazole ring str); $^1$H NMR (500 MHz, DMSO-d$_6$) $\delta$ 0.95 (s, 18H, tert-butyl), 1. (s, 18H, tert-butyl), 3.84 (d, 4H, $J$=12.8 Hz, Ar-CH$_2$-Ar), 4.17 (d, 4H, $J$=13.15 Hz, Ar-CH$_2$-Ar), 4.12 (s, 4H, CH$_2$O), 6.70-7.25 (m, 8H, ArH), 8.10 (s, 2H, OH), 7.35-9.24 (s, 8H, PyH), 10.10 (d, 2H, NH),
$^{13}$C NMR (125 MHz, DMSO-$d_6$) δ 31.4, 32.9, 34.2, 33.4 (Me$_3$C), 32.3 (Ar-CH$_2$-Ar), 122.4, 125.9, 127.4, 134.2, 143.7, 146.3, 149.1, 150 (CH of Ar), 167.4 (NHCO of amide), 66.40 (OCH$_2$), 122.3-168.4 (C1-C5 of pyridine ring), 152.5-166.1 (C6-C7 of thiadiazole ring); FAB-MS (m/z) 1084 (M+1).

2.3.4.8 5,11,17,23-tetra-tert-butyl-25,27-bis(5-(styryl)-1,3,4-oxadiazole-2-thiocarbonylmethoxy)-26,28-dihydroxycalix[4]arene (5h):

Yield 83%; mp > 250 °C; Anal. Calcd for C$_{64}$H$_{66}$N$_6$O$_6$S$_2$ (1137.45): C, 71.80; H, 6.38; N, 4.93; O, 11.25; S, 5.64. Found: C, 71.92; H, 6.28; N, 4.82; O, 11.33; S, 5.60%. FT-IR (KBr, ν cm$^{-1}$), 3440 (-OH), 3010 (-Ar-CH), 1715 (-C=O), 1735, 1012 (C-O-C linkage), 1610, 1404, 1340 (oxadiazole ring str); $^1$H NMR (500 MHz, DMSO-$d_6$) δ 0.96 (s, 18H, tert-butyl), 0.98 (s, 18H, tert-butyl), 3.90 (d, 4H, $J$=12.7 Hz, Ar-CH$_2$-Ar), 4.25 (d, 4H, $J$=12.9 Hz, Ar-CH$_2$-Ar), 4.4 (s, 4H, OCH$_2$), 6.59-7.24 (m, 8H, ArH), 8.10 (s, 2H, OH), 7.24-8.28 (s, 8H, PyH), $^{13}$C NMR (125 MHz, DMSO-$d_6$) δ 31.3, 32.9, 34.5, 33.4 (Me$_3$C of tert-butyl), 33.8 (Ar-CH$_2$-Ar), 121.2, 125, 126.4, 133.2, 144.7, 147.3, 150.2 (CH of Ar), 161.5 (SCO), 65.4 (OCH$_2$), 123.3-158.2 (C1-C5 of pyridine ring), 161.5-169 (C6-C7 of oxadiazole ring); FAB-MS (m/z) 1138 (M+1).

2.3.4.9 5,11,17,23-tetra-tert-butyl-25,27-bis(5-(styryl)-1,3,4-thiadiazole-2-aminemethyleneoxy)-26,28-dihydroxycalix[4]arene (5i):

Yield 82%; mp > 250 °C; Anal. Calcd for C$_{64}$H$_{68}$N$_8$O$_6$S$_2$ (1135.48): C, 71.93; H, 6.57; N, 7.40; O, 8.45; S, 5.65. Found: C, 71.90; H, 6.54; N, 7.42; O, 8.48; S, 5.64%. FT-IR (KBr, ν cm$^{-1}$), 3411 (-OH), 29285 (-Ar-CH), 1640-1661 (NHCO), 673 (C-S-C linkage), 1232 (N-N=C thiadiazole ring str); $^1$H NMR (500 MHz, DMSO-$d_6$), δ 0.94 (s, 18H, tert-butyl), 1.00 (s, 18H, tert-butyl), 3.84 (d, 4H, $J$=12.8 Hz, Ar-CH$_2$-Ar), 4.16 (d, 4H, $J$=13.15 Hz, Ar-CH$_2$-Ar), 4.41 (s, 4H, OCH$_2$), 6.75-7.28 (m, 8H, ArH), 8.12 (s, 2H, OH), 7.25-9.24 (s, 8H, pyH), 10.13 (d, 2H, NH); $^{13}$C NMR (125 MHz, DMSO-$d_6$) δ 31.2, 32.9, 34.2, 33.4 (Me$_3$C), 32.3 (Ar-CH$_2$-Ar), 121.4, 125.9, 127.4, 134.2, 143.7, 146.3, 149.1, 153 (CH of Ar), 166.4 (NHCO of amide), 63.4 (OCH$_2$), 125.3-168.4 (C1-C5 of pyridine ring), 151.5-168.1 (C6-C7 of thiadiazole ring); FAB-MS (m/z) 1136 (M+1).
2.4 Biological Assay

2.4.1 In vitro evaluation of antimicrobial activity

The Minimum Inhibitory Concentration (MIC) of synthesized compounds A-I, and 5a-5i was carried out by broth micro dilution method as described by Rattan [59]. Antibacterial activity was screened against two +ve (Gram positive) bacteria (S. aureus MTCC 96, S. pyogenes MTCC 442) and two -ve (Gram negative) bacteria (E. coli MTCC 443, P. aeruginosa MTCC 1688). Ampicillin was used as a standard antibacterial agent. Antifungal activities of the synthesized compounds were screened against two fungal species (C. albicans MTCC 227, A. clavatus MTCC 1323). Ampicillin and Griseofulvin were used as standard drugs.

All MTCC (Microbial Type Culture Collection) cultures were provided by the Institute of Microbial Technology, Chandigarh India and tested against standard known drugs ampicillin and griseofulvin. Mueller–Hinton broth was used as the nutrient medium to grow and to dilute the drug suspension for the test. Inoculum size for test strain was adjusted to $10^8$ (Colony Forming Unit) CFU per milliliter by comparing the turbidity. Dimethyl suloxide (DMSO) was used as diluents to get desired concentration of drugs to test upon standard bacterial strains. Serial dilutions were prepared for primary and secondary screening. The control tube, containing no antibiotic, was immediately subcultured (before inoculation) by spreading a loopful evenly over a quarter of plate of a medium suitable for the growth of the test organism and put for incubation overnight at 37 °C. The MIC of the control organism was measured to check the accuracy of the drug concentrations. The lowest concentration inhibiting growth of the organism was recorded as the MIC. All the tubes not showing visible growth (in the same manner as a control tube described above) was subcultured and incubated overnight at 37 °C. The amount of growth from the control tube before incubation (which represents the original inoculum) was compared. Subcultures might show similar number of colonies indicating bacteriostatic, a reduced number of colonies indicating a partial or slow bactericidal activity and no growth if the whole inoculum has been killed. The test included a second set of the same dilutions inoculated with an organism of known sensitivity. A stock solution of 2000 μg/mL of each of the synthesized compounds was prepared and diluted as and when required. In primary screening 500, 250, 200 and 125 μg/mL concentrations
of the synthesized drugs were taken. The active synthesized drugs found in this primary screening were further tested in a second set of dilution against all microorganisms. The drugs found active in primary screening were similarly diluted to obtain 100, 50, 25, 12.5, 6.25, 3.125 and 1.5625 μg/mL concentrations. The highest dilution showing at least 99% inhibition is taken as MIC.

2.4.2 In vitro evaluation of antimycobacterial activity

Drug susceptibility and determination of MIC of the test compounds against *M. tuberculosis* H37Rv were performed by Lowenstein-Jensen (LJ) MIC method [60-63] where primary 1000, 500, 250, and secondary 200, 100, 62.5, 50, 25, 12.5, 6.25, 3.25 μg/mL dilutions of each test compound was added to liquid Lowenstein-Jensen medium and then media were sterilized by inspissations method. A culture of *M. tuberculosis* H37Rv growing on Lowenstein-Jensen medium was harvested in 0.85% saline in bijou bottles. Solution of 2000 mg/L concentration of each test compounds was prepared in (DMSO). These tubes were then incubated at 37 °C for 24 h followed by streaking of *M. tuberculosis* H37Rv (5x10⁴ bacilli per tube). These 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 no development of colonies occurred or < 20 colonies was taken as MIC concentration of test compound. The standard strain *M. tuberculosis* H37Rv was tested with known drug isoniazid.

2.4.3 Antioxidant activity

Free radical scavenging activity of the tested compounds 5a-5i was studied by the (diphenyl picryl hydrazyl) DPPH assay method. [63] The drug stock solution (1 mg/mL) was diluted to final concentrations of 2, 4, 6, 8 and 10 μg/mL in methanol. DPPH in methanol solution (1 mL, 0.3 mmol) was added to 2.5 mL of drug solutions of different concentrations and were then allowed to react at room temperature. After 30 min, the absorbance values were measured at 518 nm and were converted into the percentage antioxidant activity. Methanol was used as the solvent and ascorbic acid as the standard. The trials were performed in triplicate.
2.5 Results and Discussion

The antibacterial activities (Figure 7), and antifungal activity (Figure 8), of compounds A-I, and 5a-5i are presented in Table 1 & Table 2. The results revealed that the compounds A and I displayed good activity compared to others against Gram -ve bacteria E.coli (growth inhibition zones 14 ± 0.04 and 12.10 ± 0.02 mm) at MIC 200 µg/mL and compounds A and D showed good inhibition compared to others against Gram -ve bacteria P.aeruginosa (growth inhibition zones 13 ± 0.02 and 13.00 ± 0.43 mm) at MIC 200 µg/mL, also compound A showed good inhibition against Gram +ve S.aureus (% inhibition 13 ± 0.02 mm) at MIC 100 µg/mL and compound A showed good inhibition against gram +ve S.pyogenes (growth inhibition zones 14.50 ± 0.03 mm) at MIC 200 µg/mL. The compounds 5c, 5h and 5b displayed excellent activity against both Gram -ve bacteria E.coli (growth inhibition zones 18 ± 0.02, 17.32 ± 0.29 and 17 ± 0.01 mm) at MIC 100 µg/mL and P.aeruginosa (growth inhibition zones 18 ± 0.02, 18.00 ± 0.25 and 16.50 ± 0.15 mm) at MIC 100 µg/mL. Compounds 5a and 5h exhibited excellent activity against S.aureus Gram +ve bacteria (growth inhibition zones 17 ± 0.02 and 17.30 ± 0.78 mm) at MIC 100 µg/mL and S.pyogenes (gram +ve bacteria) (growth inhibition zones 19 ± 0.03 and 19.00 ± 0.47) at MIC 200 µg/mL. The compounds 5d, 5f and 5i displayed moderate to good inhibition zones against both Gram -ve bacteria (growth inhibition zones 17.35 ± 0.18 and 16.40 ± 0.29, 15.10 ± 0.02 mm) at MIC 200 µg/mL whereas compounds 5b and 5c also good activity against both Gram +ve bacteria (growth inhibition zones 16 ± 0.05, and 16 ± 0.03 mm) at MIC 200 µg/mL and (growth inhibition zones 17 ± 0.04 and 18 ± 0.04 mm) at MIC 250 µg/mL. The compounds 5e and 5g showed weak activity against E.coli bacteria (growth inhibition zones < 12.00 ± 0.74, 12.40 ± 0.03 mm) at MIC 250 µg/mL while compound 5g showed weak activity against both Gram +ve bacteria (growth inhibition zones 11.00 ± 0.15 and 14.55 ± 0.99 mm) at MIC 250 µg/mL respectively. Compounds A and D displayed moderate to good inhibition zone against C.albicans (growth inhibition zones 16.24 ± 0.32 and 14.36 ± 0.65 mm) at MIC 250 µg/mL, It showed good inhibition against A. clavatus (growth inhibition zones 16.37 ± 0.31 mm) at MIC 500 µg/mL. All the tested compounds 5a, 5d and 5h displayed excellent activity against fungus C.albicans (growth inhibition zones 23.30 ± 0.02, 22.36 ± 0.79 and 21.36 ± 0.79 mm) at MIC 250 µg/mL while compound
5h exhibited excellent activity against fungi *A. clavatus* (growth inhibition zones 22 ± 0.01) at MIC 250 μg/ml. Compounds 5c, 5b, 5f and 5i showed moderate to good inhibitory effect towards tested fungus (growth inhibition zones > 17 mm) at MIC 500 μg/ml. Compounds 5e and 5g showed weak inhibitory effect towards *C. albicans* (growth inhibition zones 12.07 ± 0.22, and 9 ± 0.07 mm) at MIC 500 μg/mL and 5g exhibit weak activity against *A. clavatus* (growth inhibition zones 11.30 ± 0.12 mm) at MIC 500 μg/mL.

*In vitro* antimycobacterial activity (Figure 9) showed that all the compounds are bioactive against *M. tuberculosis H₃₇Rv* comparable to standard drugs, compounds A, C and H showed good anti-mycobacterial activity (% inhibition 82.27 ± 0.04, 71.50 ± 0.03 and 63.23 ± 0.90) at MIC 50 μg/mL and 100 μg/mL. Compounds 5a and 5c also exhibited excellent anti mycobacterial activity (% inhibition 94 ± 0.01, 96.54 ± 0.90) at MIC 50 μg/mL and 62.5 μg/mL against *M. tuberculosis H₃₇Rv*. The compounds 5b, 5d and 5h showed moderate to good antimycobacterial activity (% inhibition 92.80 ± 0.07, 89.42 ± 0.8 and 91.23 ± 0.92) at MIC 150, 100 & 62.5 μg/mL. Compounds 5f, 5i and 5e displayed less mycobacterial inhibition (% inhibition 45 ± 0.06, 57.10 ± 0.41 and 38.24 ± 0.57,) at MIC 200, μg/mL towards *M. tuberculosis H₃₇Rv*. The compound 5g showed no inhibition displayed in (Table 3). The presence of the 1,3,4-oxadiazole and 1,3,4-thiadiazole ring as pharmacophores and increase in the lipophilic character of the molecule due to the presence of substituted calix[4]arene in the molecule which facilitate the crossing through the biological membrane of the micro-organism there by inhibiting their growth.

Free radical scavenging activity of the tested compounds 5a-5i was studied by the DPPH assay method. The inhibitory concentration value, represent the concentration required to exhibit 50% antioxidant activity (IC₅₀) (Table 4). In the synthesized compounds, compounds 5h & 5c were found to possess maximum antioxidant activity 82.6%, 75.0% and their inhibitory concentration (IC₅₀ : 6.1 μg/mL, IC₅₀ : 6.45 μg/mL) against the standard drug ascorbic acid 92 % (IC₅₀:5.7 μg/mL). However, compound, 5f showed moderate antioxidant activity 68.4 % (IC₅₀ : 7.42 μg/mL), whereas 5b & 5g showed minimum antioxidant activity (IC₅₀ >10 μg/mL). The results revealed that the
compounds having an oxadiazole moiety exhibited good antioxidant property, compared to those having a thiadiazole moiety. Results are presented in (Figure 10, 11).

2.6 Conclusion

We can conclude from the above investigations that all the synthesized novel calix[4]arene assembly incorporating 1,3,4-oxadiazole and 1,3,4-thiadiazole heterocycles were efficient against the tested Gram negative (E. coli, P. aeruginosa), Gram positive (S. aureus, S. pyogenes) strains, two fungi species (C. albicans, A. clavatus) and H37Rv bacteria. Their activities were comparable to standard drugs. Compound 5a 5,11,17,23-tetra-tert-butyl-25,27-bis(5-(pyridin-4-yl)-1,3,4-oxadiazole-2-thiacarbonyl-methoxy)-26,28-dihydroxycalix[4]arene and 5c 5,11,17,23-tetra-tert-butyl-25,27-bis(5-(pyridin-4-yl)-1,3,4-oxadiazole-2-amidemethylene oxy)-26,28-dihydroxycalix[4]arene exhibited better bioactivity, especially towards M. tuberculosis against standard drug (Isonazid). The presence of the 1,3,4-oxadiazole and thiadiazole ring as pharmacophores and the increase in the lipophilic character of the molecule due to the presence of substituted calix[4]arene in the molecule facilitated the crossing through the biological membrane of the micro-organism thereby inhibiting their growth. Moreover, the results also confirm the organizational role of calixarene in bringing together the oxadiazole groups for the genesis of anti-mycobacterial activity. The enhancement of activity of oxadiazole and thiadiazole functionalized calix[4]arene compared to the unsubstituted oxadiazole and thiadiazole (A-I) is attributed to the cooperative effect of the pharmacophores. Further, more compounds having an oxadiazole moiety showed good antioxidant activity.
FIGURES

Figure 7. Antibacterial activity of compounds (A-I) and (5a-5i).

Figure 8. Antifungal activity of compounds (A-I) and (5a-5i).
Figure 9. Antifungal activity of compounds (A-I) and (5a-5i).

Figure 10. Antioxidant activity of compounds (5a-d).

Figure 11. Antioxidant activity of compounds (5e-i).
Table 1.

*In vitro* antibacterial activity of compounds (A-I) and newly synthesized calix[4]arene based 1,3,4-oxadiazoles and 1,3,4-thiadiazole derivatives of isoniazid, nicotinic acid hydrazide, benzoic acid hydrazide and cis-cinnamic acid hydrazide (5a-5i).

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<th>Compound No</th>
<th>Concentration (μg/disc)</th>
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<th><em>S. aureus</em></th>
<th><em>S. pyogenes</em></th>
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<td>13.00 ± 0.02 (100)</td>
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Table 2.

*In vitro* antifungal activity of compounds (A-I) and the newly synthesized calix[4]arene based 1,3,4-oxadiazoles and 1,3,4-thiadiazole derivatives of isoniazid, nicotinic acid hydrazide, benzoic acid hydrazide, cis-cinnamic acid hydrazide (5a-5i).

<table>
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<th>Compound No</th>
<th>Concentration µg/disc</th>
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<th><em>A.clavatus</em></th>
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<td>A</td>
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<td>B</td>
<td>250</td>
<td>12.00 ± 0.01 (500)</td>
<td>12.33 ± 0.05 (500)</td>
</tr>
<tr>
<td>C</td>
<td>250</td>
<td>14.25 ± 0.04 (500)</td>
<td>11.45 ± 0.15 (500)</td>
</tr>
<tr>
<td>D</td>
<td>250</td>
<td>14.36 ± 0.65 (250)</td>
<td>13.00 ± 0.43 (500)</td>
</tr>
<tr>
<td>E</td>
<td>250</td>
<td>13.07 ± 0.12 (500)</td>
<td>14.35 ± 0.42 (500)</td>
</tr>
<tr>
<td>F</td>
<td>250</td>
<td>10.10 ± 0.15 (500)</td>
<td>14.00 ± 0.05 (500)</td>
</tr>
<tr>
<td>G</td>
<td>250</td>
<td>6.50 ± 0.70 (500)</td>
<td>10.30 ± 0.12 (500)</td>
</tr>
<tr>
<td>H</td>
<td>250</td>
<td>14.30 ± 0.56 (250)</td>
<td>16.37 ± 0.31 (500)</td>
</tr>
<tr>
<td>I</td>
<td>250</td>
<td>13.20 ± 0.58 (500)</td>
<td>14.22 ± 0.25 (500)</td>
</tr>
<tr>
<td>5a</td>
<td>250</td>
<td>23.30 ± 0.02 (250)</td>
<td>24.25 ± 0.03 (500)</td>
</tr>
<tr>
<td>5b</td>
<td>250</td>
<td>21.50 ± 0.01 (500)</td>
<td>20.45 ± 0.05 (500)</td>
</tr>
<tr>
<td>5c</td>
<td>250</td>
<td>22.00 ± 0.04 (500)</td>
<td>21.50 ± 0.01 (500)</td>
</tr>
<tr>
<td>5d</td>
<td>250</td>
<td>22.36 ± 0.79 (250)</td>
<td>23.00 ± 0.23 (500)</td>
</tr>
<tr>
<td>5e</td>
<td>250</td>
<td>12.07 ± 0.22 (500)</td>
<td>18.45 ± 0.22 (500)</td>
</tr>
<tr>
<td>5f</td>
<td>250</td>
<td>18.00 ± 0.15 (500)</td>
<td>19.00 ± 0.35 (500)</td>
</tr>
<tr>
<td>5g</td>
<td>250</td>
<td>9.00 ± 0.07 (500)</td>
<td>11.30 ± 0.12 (500)</td>
</tr>
<tr>
<td>5h</td>
<td>250</td>
<td>21.36 ± 0.79 (250)</td>
<td>22.00 ± 0.01 (250)</td>
</tr>
<tr>
<td>5i</td>
<td>250</td>
<td>17.00 ± 0.48 (500)</td>
<td>18.32 ± 0.30 (500)</td>
</tr>
<tr>
<td>Griseofulvin$^b$</td>
<td>250</td>
<td>24.00 ± 0.00 (500)</td>
<td>24.00 ± 0.00 (100)</td>
</tr>
<tr>
<td>DMSO</td>
<td>250</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
### Table 3.

*In vitro* antituberculosis activity % inhibition against *M. tuberculosis H*$_{37}$*Rv* at concentration 250 µg/mL at (MIC µg/mL) of compounds (A-I) and newly synthesized calix[4]arene based 1,3,4-oxadiazoles and 1,3,4-thiadiazole derivatives of isoniazid, nicotinic acid hydrazide, benzoic acid hydrazide, cis-cinnamic acid hydrazide (5a-5i).

<table>
<thead>
<tr>
<th>Compound No</th>
<th>(MIC in µg/mL)$^a$</th>
<th>% INHIBITION</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>50</td>
<td>82.27 ± 0.04</td>
</tr>
<tr>
<td>B</td>
<td>100</td>
<td>62.00 ± 0.02</td>
</tr>
<tr>
<td>C</td>
<td>100</td>
<td>71.50 ± 0.03</td>
</tr>
<tr>
<td>D</td>
<td>100</td>
<td>62.21 ± 0.80</td>
</tr>
<tr>
<td>E</td>
<td>250</td>
<td>38.24 ± 0.57</td>
</tr>
<tr>
<td>F</td>
<td>100</td>
<td>45.00 ± 0.60</td>
</tr>
<tr>
<td>G</td>
<td>200</td>
<td>35.00 ± 0.64</td>
</tr>
<tr>
<td>H</td>
<td>100</td>
<td>63.23 ± 0.90</td>
</tr>
<tr>
<td>I</td>
<td>125</td>
<td>34.10 ± 0.51</td>
</tr>
<tr>
<td>5a</td>
<td>50</td>
<td>94.00 ± 0.01</td>
</tr>
<tr>
<td>5b</td>
<td>150</td>
<td>92.80 ± 0.07</td>
</tr>
<tr>
<td>5c</td>
<td>62.5</td>
<td>96.54 ± 0.90</td>
</tr>
<tr>
<td>5d</td>
<td>100</td>
<td>89.42 ± 0.8</td>
</tr>
<tr>
<td>5e</td>
<td>200</td>
<td>38.24 ± 1.57</td>
</tr>
<tr>
<td>5f</td>
<td>200</td>
<td>45.00 ± 0.06</td>
</tr>
<tr>
<td>5g</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5h</td>
<td>62.5</td>
<td>91.23 ± 0.92</td>
</tr>
<tr>
<td>5i</td>
<td>200</td>
<td>57.10 ± 0.41</td>
</tr>
<tr>
<td>Isoniazid$^b$</td>
<td>0.2</td>
<td>99.00</td>
</tr>
</tbody>
</table>

$^a$MIC values are given in brackets. MIC (µg/mL) = Minimum inhibitory concentration.

$^b$Standard Mean of five replicates: ± Standard deviation, (-) No activity,
Table 4.
Antioxidant data for the newly synthesized calix[4]arene based 1,3,4-oxadiazoles and 1,3,4-thiadiazole derivatives of isoniazid, nicotinic acid hydrazide, benzoic acid hydrazide and cis-cinnamic acid hydrazide.

<table>
<thead>
<tr>
<th>Compound No</th>
<th>DPPH scavenging (%)</th>
<th>IC$_{50}$ µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>5a</td>
<td>62.0</td>
<td>8.0 ± 0.05</td>
</tr>
<tr>
<td>5b</td>
<td>50.0</td>
<td>c</td>
</tr>
<tr>
<td>5c</td>
<td>75.0</td>
<td>6.45 ±0.11</td>
</tr>
<tr>
<td>5d</td>
<td>72.3</td>
<td>7.23 ±0.1</td>
</tr>
<tr>
<td>5e</td>
<td>54.6</td>
<td>9.34 ±0.17</td>
</tr>
<tr>
<td>5f</td>
<td>68.4</td>
<td>7.42 ±0.04</td>
</tr>
<tr>
<td>5g</td>
<td>48.6</td>
<td>c</td>
</tr>
<tr>
<td>5h</td>
<td>82.6</td>
<td>6.1 ± 0.04</td>
</tr>
<tr>
<td>5i</td>
<td>64.4</td>
<td>8.16 ± 0.10</td>
</tr>
<tr>
<td>Ascorbic acid$^b$</td>
<td>92.0</td>
<td>5.70 ± 0.00</td>
</tr>
</tbody>
</table>

$^a$ Results are mean of three different experiment.

$^b$ Standard

Mean of three replicates: ± Standard deviation

$^c$ Low antioxidant activity (IC$_{50}$ > 10 µg/mL)
References


