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PAPER

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

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In the present investigation, we describe some novel calixarene based heterocyclic compounds (**5a–5i**) in which 1,3,4-oxadiazole and 1,3,4-thiadiazole derivatives have been coupled with 5,11,17,23-tetra-*tert*-butyl-25,27-bis(chlorocarbonyl-methoxy)-26,28-dihydroxy calix[4]arene. All the newly synthesized calixarene based heterocyclic compounds have been characterized by elemental analysis and various spectroscopic methods like FTIR, ^{1}H NMR, ^{13}C NMR, and FAB-MS. All the final scaffolds have been subjected to antioxidant activity, *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*) and also have been screened for their antitubercular activity against *Mycobacterium tuberculosis* $H_{37}Rv$.

Introduction

The development of biologically active molecules based on molecular recognition is an attractive and challenging research topic in medicinal and supramolecular chemistry.[1] The calixarenes have often been employed in recent years as carriers and spatial organizers of various kinds of active substituent, displaying properties dealing with recognition of organic substrates or metallic cations. Efforts have also been made to use their specific conformations to prepare, notably in the case of the tensed calix[4]arene, highly ordered and organized molecular devices.[2–5] As assessed by recent reviews,[6–10] the calixarenes have been investigated in the medicinal field, some patents, have been devoted to their use in this domain, for example, hydrophilic derivatives have shown interesting levels of activity against bacteria,[11] fungi, cancerous cell, enveloped virus,[12] thrombosis [13] and fibrosis diseases.[14]

In the mid-1950s, the calixarene derivative 'Macrocyclon' [15] and more recently some parent structures [16,17] have been studied in the treatment of tuberculosis and other mycobacterioses.

The building of designed calixarenic mimics of vancomycin has also been studied, with the aim of focusing on an antimicrobial activity,[18] and some biological studies related to plasmid DNA binding, and cell transfection have notably been reported by Ungaro and co-workers.[19–21] Regnouf and coworkers, focusing on the development of calixarene platforms designed molecular drug dispensers offering penicillin and quinolone moieties at the lower rim.[22–25] Ionic calixarene derivatives exhibit intrinsic antimicrobial activity,[26–29] while *p*-guanidino ethyl calixarene and parent phenol derivatives exhibited antibacterial activities.[30] Hydroxycinnamic acid based derivatives also had been reported as radical scavenging agents having antioxidant activity.[31]

Heterocyclic compounds containing five-membered rings gained importance because of their versatile biological properties. In particular, compounds bearing 1,3,4-oxadiazole nucleus has been found to exhibit diverse biological activities such as antimicrobial,[32–34] anti-HIV,[32] antitubercular,[35,36] antioxidant [37] and antimalarial.[38]

The emergence of resistance in pathogenic microorganisms to commercial antibiotic calls for the development of specific fields of research dedicated to the discovery of new drugs.[39] Tuberculosis (TB) is one of the most common infectious diseases known to mankind. Around 32% of the world's population is infected by *Mycobacterium tuberculosis*, the main causal agent of TB. Problems in the treatment of tuberculosis arise when bacteria develop resistance to the first-line drugs like isoniazid, rifampicin, ethambutol, streptomycin, and pyrazinamide.[40]

Isoniazid is a prodrug and must be activated by bacterial catalase. It is activated by catalase–peroxidase hemoproteins, KatG, which couples the isonicotinic acyl with nicotinamide adenine dinucleotide (NADH) to form the isonicotinic acyl-NADH complex. This complex indirectly inhibits the synthesis of mycolic acid required for the mycobacterial cell wall.[41] It is bactericidal to rapidly-dividing mycobacteria but is bacteriostatic if the *Mycobacterium* is slow-growing.[42] Isoniazid is metabolized by the liver, mainly by acetylation and dehydrogenation. The

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N-acetyl hydrazine metabolite is believed to be responsible for the hepatotoxicity effects seen in patients treated with isoniazid. It is established that CYP2E1 (cytochrome P450, family 2, subfamily E polypeptide 1) is reportedly involved in INH-induced hepatotoxicity.[43]

For this reason, mentioned above in our study, we tried to eliminate *in vivo* acetylation by arylamine *N*-acetyltransferase (NAT) to form an inactive acetylated drug, by replacing the hydrazide moiety of INH (isonicotinic acid hydrazide) with 1,3,4-thiadiazole and 1,3,4-oxadiazole heterocycles.

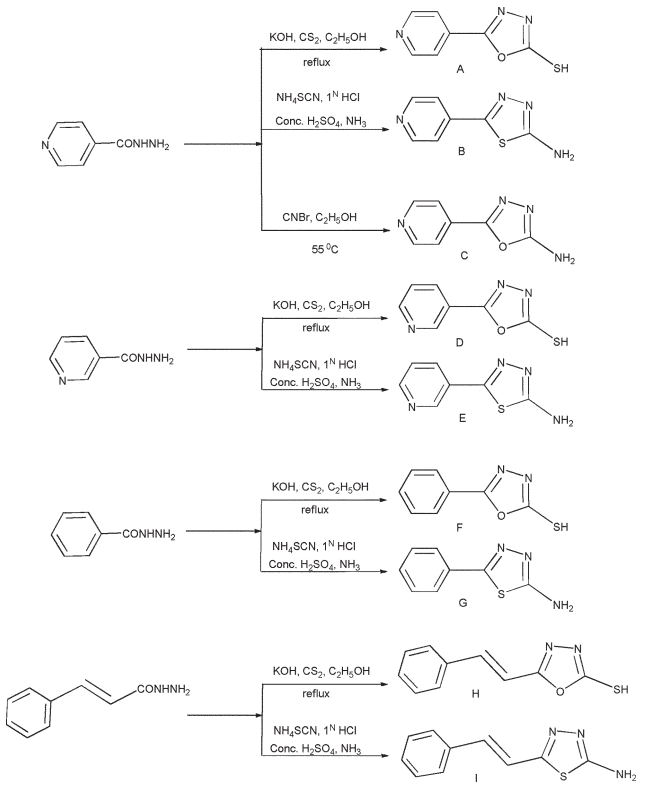
We report here the synthesis and characterization of novel calix[4]arene derivatives incorporating two oxadiazole and thiadiazole subunits at the lower rim. The pharmacophores oxadiazole and thiadiazole subunits were incorporated into functionalized calix[4]arene anticipating an enhancement of lipophilicity and a cooperative effect of the subunits by bringing them together at the lower rim, resulting from the organizational role of the calixarene core. We also have evaluated their antioxidant activity, antibacterial activity against gram +ve and gram −ve strains, antifungal activity against fungal strains and antitubercular activity against $H_{37}R_V$ bacteria.

Results and discussion

Chemistry

As part of our research program on developing new antimycobacterial and antimicrobial agents, we designed several functionalized fullerenes,[44,45] and now the work is extended to functionalized calixarenes. For the synthesis of functionalized calixarenes the hydrazides of nicotinic acid, benzoic acid and *cis*-cinnamic acid were prepared by refluxing the above acids with sulphuric acid and methanol for 10 h to form the corresponding acid ester and this ester was condensed with hydrazine hydrate by maintaining the reaction temperature at 0 °C to form nicotinic acid, benzoic acid and *cis*-cinnamic acid hydrazide. Intramolecular cyclization of nicotinic acid, benzoic acid and *cis*-cinnamic acid hydrazide with carbon disulfide and potassium hydroxide in the presence of ethanol resulted in 5-(substituted pyridyl)-1,3,4-oxadiazole-2-thiols (**A**, **D**, **F** & **H**). The corresponding acid hydrazide was refluxed with ammonium thiocyanate in an acidic medium to obtain acid thiosemicarbazides, which gave 2-amino-5-(substituted pyridyl)-1,3,4-thiadiazoles (**B**, **E**, **G** & **I**) on cyclization in the presence of conc. sulfuric acid. Isoniazid, on stirring and heating with cyanogen bromide, gave 2-amino-5-(pyridin 4-yl)-1,3,4-oxadiazole (**C**). (Scheme 1)

The tetra-*tert*-butylcalix[4]arene **1** was prepared according to Gutche's procedure.[46] The synthesis of 5,11,17,23-*tetra-tert*-butyl-25,27-diethoxycarbonylmethoxy-26,28-dihydroxy calix-[4]arene **2** was carried out by the reaction of tetra-*p-tert*-butylcalix[4]arene **1** and bromo ester in refluxing acetone in the presence of K_2CO_3 as a base, and gave 78% yield. Ester **2** was hydrolyzed in a hydroalcoholic medium to give the corresponding diacid **3**, with 97% yield. The diacid **3**, when refluxed in the presence of thionyl chloride, afforded the corresponding diacid chloride compound **4** with a quantitative yield. Compound **4** was then refluxed in THF with compound (**A**, **D**, **F** & **H**) in the presence of pyridine to give the corresponding 1,3,4-oxadiazole coupled calix[4]arenes (**5a**, **5d**, **5f** & **5h**). Compound **4** when refluxed in THF with the 2-amino-5-(substituted pyridyl)-1,3,4-thiadiazoles compounds (**B**, **E**, **G** & **I**) in a presence of pyridine gave the corresponding 1,3,4-thiadizole coupled calix[4]arenes (**5b**, **5e**, **5g** & **5i**), Compound **4** when refluxed in THF with **C** in the presence of pyridine gave corresponding 5,11,17,23-tetra-*tert*-butyl-25,27-bis(5-(pyridin-4-yl)-1,3,4-oxadiazole-2-amidemethyleneoxy) 26,28-dihydroxy calix[4]arene **5c**. (Scheme 2)



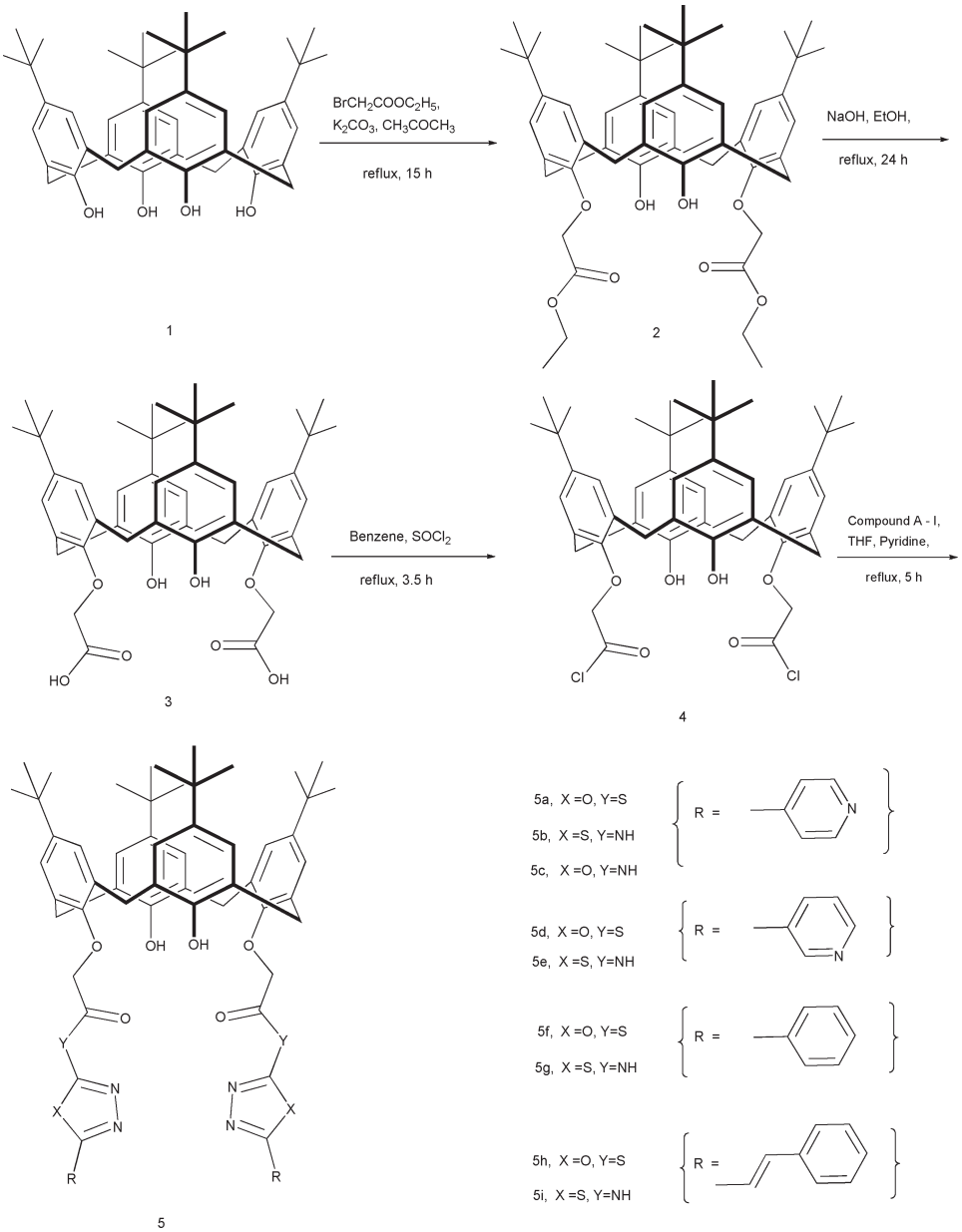
Scheme 1 Synthesis of oxadiazole & thiadiazole derivatives of isoniazid, nicotinic, benzoic and *cis*-cinnamic acid hydrazide.

Biological evaluation

In vitro evaluation of antimicrobial activity

The Minimum Inhibitory Concentration (MIC) of synthesized compounds **5a–5i** was carried out by broth micro dilution method as described by Rattan.[47] 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. The antifungal activity of the synthesized compounds **5a–5i** was 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 and tested against 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



Scheme 2 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.

test strain was adjusted to 10^8 CFU (Colony Forming Unit) per millilitre by comparing the turbidity. 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 read 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, or 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^{-1} of each of the synthesized compounds was prepared and diluted as and when required. In the primary screening 500, 250, 200 and 125 μg mL^{-1} 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, 6.250, 3.125 and 1.5625 μg mL^{-1} concentrations. The highest dilution showing at least 99% inhibition is taken as MIC.

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**)

In vitro activity-zone of inhibition in mm (MIC in μg mL^{-1})[a]					
		Gram+ve		*Gram−ve*	
Compound no	Concentration μg/disc	*E.coli* MTCC 443	*P. aeruginosa* MTCC 1688	*S. aureus* MTCC 96	*S. pyogenes* MTCC 442
A	250	14.00 ± 0.04 (200)	13.00 ± 0.02 (200)	13.00 ± 0.02 (100)	14.50 ± 0.03 (200)
B	250	12.00 ± 0.01 (250)	12.40 ± 0.04 (200)	14.50 ± 0.05 (200)	13.00 ± 0.03 (200)
C	250	11.00 ± 0.13 (250)	12.67 ± 0.01 (250)	14.00 ± 0.02 (250)	12.00 ± 0.44 (250)
D	250	10.35 ± 0.18 (250)	13.00 ± 0.43 (200)	11.00 ± 0.03 (250)	10.40 ± 0.20 (250)
E	250	10.50 ± 0.34 (250)	11.45 ± 0.94 (200)	12.00 ± 0.25 (250)	10.25 ± 0.50 (250)
F	250	11.40 ± 0.29 (250)	12.15 ± 0.95 (250)	11.25 ± 0.40 (200)	11.12 ± 0.40 (250)
G	250	8.00 ± 0.74 (500)	8.40 ± 0.03 (200)	8.30 ± 0.15 (250)	6.55 ± 0.90 (250)
H	250	11.34 ± 0.59 (250)	12.00 ± 0.45 (250)	14.30 ± 0.78 (250)	13.00 ± 0.57 (250)
I	250	12.10 ± 0.02 (200)	11.50 ± 0.35 (250)	12.40 ± 0.15 (250)	13.20 ± 0.60 (250)
5a	250	19.00 ± 0.01 (200)	19.00 ± 0.02 (200)	17.00 ± 0.02 (100)	19.00 ± 0.03 (200)
5b	250	17.00 ± 0.01 (100)	18.00 ± 0.04 (200)	16.00 ± 0.05 (200)	16.00 ± 0.03 (200)
5c	250	18.00 ± 0.02 (100)	18.00 ± 0.02 (100)	17.00 ± 0.04 (250)	18.00 ± 0.04 (250)
5d	250	17.35 ± 0.18 (200)	19.00 ± 0.43 (200)	16.00 ± 0.33 (100)	17.40 ± 0.02 (250)
5e	250	12.20 ± 0.34 (250)	17.46 ± 0.94 (250)	15.00 ± 0.25 (250)	15.00 ± 0.05(200)
5f	250	16.40 ± 0.29 (200)	17.15 ± 0.95 (200)	14.20 ± 0.40 (100)	15.15 ± 0.68 (200)
5g	250	12.00 ± 0.74 (250)	12.40 ± 0.03 (250)	11.00 ± 0.15 (250)	14.55 ± 0.90 (250)
5h	250	17.32 ± 0.29 (100)	18.00 ± 0.25 (100)	17.30 ± 0.78 (100)	19.00 ± 0.47 (200)
5i	250	15.10 ± 0.02 (200)	16.50 ± 0.15 (100)	13.00 ± 0.15 (200)	16.30 ± 0.60 (200)
Ampicillin[b]	250	19.00 ± 0.00 (100)	20.00 ± 0.00 (100)	18.00 ± 0.00 (250)	19 ± 0.00 (100)
DMSO	250	—	—	—	—

[a] MIC values are given in brackets. MIC (μg mL^{-1}) = Minimum inhibitory concentration, mean of five replicates ± standard deviation. [b] Standard

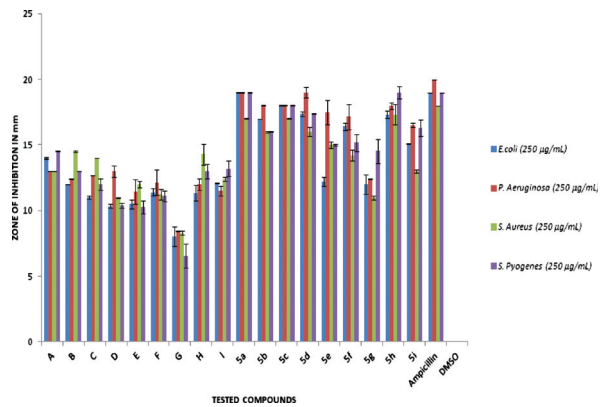


Fig. 1 Antibacterial activity of compounds (**A–I**) and (**5a–5i**).

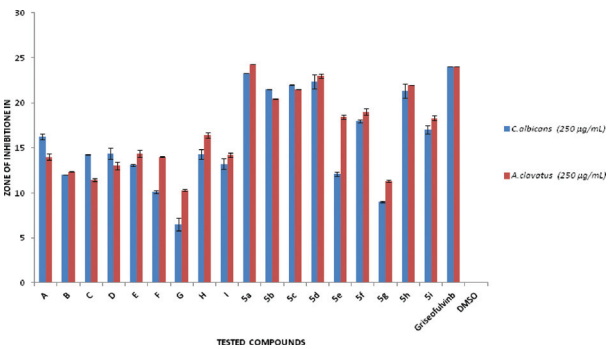


Fig. 2 Antifungal activity of compounds (**A–I**) and (**5a–5i**).

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**)

In vitro activity-zone of inhibition in mm (MIC in μg mL^{-1})[a]			
Compound no	Concentration μg/disc	*C.albicans* MTCC 227	*A.clavatus* MTCC 1323
A	250	16.24 ± 0.32 (250)	14.00 ± 0.35 (500)
B	250	12.00 ± 0.01 (500)	12.33 ± 0.05 (500)
C	250	14.25 ± 0.04 (500)	11.45 ± 0.15 (500)
D	250	14.36 ± 0.65 (250)	13.00 ± 0.43 (500)
E	250	13.07 ± 0.12 (500)	14.35 ± 0.42 (500)
F	250	10.10 ± 0.15 (500)	14.00 ± 0.05 (500)
G	250	6.50 ± 0.70 (500)	10.30 ± 0.12 (500)
H	250	14.30 ± 0.56 (250)	16.37 ± 0.31 (500)
I	250	13.20 ± 0.58 (500)	14.22 ± 0.25 (500)
5a	250	23.30 ± 0.02 (250)	24.25 ± 0.03 (500)
5b	250	21.50 ± 0.01 (500)	20.45 ± 0.05 (500)
5c	250	22.00 ± 0.04 (500)	21.50 ± 0.01 (500)
5d	250	22.36 ± 0.79 (250)	23.00 ± 0.23 (500)
5e	250	12.07 ± 0.22 (500)	18.45 ± 0.22 (500)
5f	250	18.00 ± 0.15 (500)	19.00 ± 0.35 (500)
5g	250	9.00 ± 0.07 (500)	11.30 ± 0.12 (500)
5h	250	21.36 ± 0.79 (250)	22.00 ± 0.01 (250)
5i	250	17.00 ± 0.48 (500)	18.32 ± 0.30 (500)
Griseofulvin[b]	250	24.00 ± 0.00 (500)	24.00 ± 0.00 (100)
DMSO	250	—	—

[a] MIC values are given in brackets. MIC (μg mL^{-1}) = Minimum inhibitory concentration. Mean of five replicates: ± Standard deviation. [b] Standard

Reviewing of the antibacterial activities (Fig. 1), and antifungal activity (Fig. 2), of compounds **A–I**, and **5a–5i** are presented in Table 1 & Table 2. The results revealed that compounds **A** and **I** displayed good activity compare 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^{-1} and compound **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^{-1}, also compound **A** showed good inhibition against gram +ve *S.aureus* (% inhibition 13 ± 0.02 mm) at MIC 100 μg mL^{-1} and compound **A** showed good inhibition against gram +ve *S.pyogenes* (growth inhibition zones 14.50 ± 0.03 mm) at MIC 200 μg mL^{-1}. 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^{-1} and *P.aeruginosa* (growth inhibition zones 18 ± 0.02, 18.00 ± 0.25 and 16.50 ± 0.15 mm) at MIC 100 μg mL^{-1}. Compounds **5a** & **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^{-1} and *S.pyogenes* (gram +ve bacteria) (growth inhibition zones 19 ± 0.03 and 19.00 ± 0.47) at MIC 200 μg mL^{-1}. Compounds **5d**, **5f** and **5i** displayed a moderate to good inhibition zone 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^{-1} and compounds **5b** and **5c** also showed good activity against both gram +ve bacteria (growth inhibition zones 16 ± 0.05, and 16 ± 0.03 mm) at MIC 200 μg mL^{-1} and (growth inhibition zones 17 ± 0.04 and 18 ± 0.04 mm) at MIC 250 μg mL^{-1}. Compounds **5e** and **5g**, showed weak activity against *E.coli* (gram −ve) bacteria (growth inhibition zones <12.00 ± 0.74, 12.40 ± 0.03 mm) at MIC 250 μg mL^{-1} 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^{-1} respectively. Compounds **A** and **D** displayed a 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^{-1}, and also compound **H** showed good inhibition against *A. clavatus* (growth inhibition zones 16.37 ± 0.31 mm) at MIC 500 μg mL^{-1}. All the tested compounds **5a**, **5d**, and **5h** displayed excellent activity against fungi *C.albicans* (growth inhibition zones 23.30 ± 0.02, 22.36 ± 0.79 and 21.36 ± 0.79 mm) at MIC 250 μg mL^{-1} while compound **5h**, exhibited excellent activity against fungi *A.clavatus* (growth inhibition zones 22 ± 0,01) at MIC 250 μg ml^{-1}. Compounds **5c**, **5b**, **5f** and **5i** showed moderate to good inhibitory effects towards tested fungi (growth inhibition zones >17 mm) at MIC 500 μg ml^{-1}. 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^{-1} and **5g** exhibited weak activity against *A.clavatus* (growth inhibition zones 11.30 ± 0.12 mm) at MIC 500 μg mL^{-1}.

In vitro evaluation of antimycobacterial activity

Drug susceptibility and determination of MIC of the test compounds against *Mycobacterium tuberculosis* $H_{37}Rv$ were performed by Lowenstein–Jensen (LJ) MIC method [48–51] where primary 1000, 500, 250, and secondary 200, 100, 62.5, 50, 25, 12.5, 6.25, 3.25 μg mL^{-1} dilutions of each test compound was added to liquid Lowenstein–Jensen medium and then media were sterilized by inspissation. A culture of *M. tuberculosis* $H_{37}Rv$ growing on Lowenstein–Jensen medium was harvested in 0.85% saline in bijou bottles. A solution of 2000 mg L^{-1} concentration of each test compound was prepared in dimethyl sulfoxide (DMSO). These tubes were then incubated at 37 °C for 24 h followed by streaking of *M.tuberculosis* $H_{37}Rv$ (5×10^4 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 the medium alone was incubated with *M.tuberculosis H37Rv*. The concentration at which no development of colonies occurred or <20 colonies was taken as the MIC concentration of the test compound. The standard strain *M. tuberculosis* $H_{37}Rv$ was tested with known drug isoniazid.

In vitro antimycobacterial activity (Fig. 3) showed that all the compounds are bioactive against *M. tuberculosis* $H_{37}Rv$ comparable to the standard. Compounds **A**, **C** and **H** showed good antimycobacterial activity (% inhibition 82.27 ± 0.04, 71.50 ± 0.03 and 63.23 ± 0.90) at MIC 50 μg mL^{-1} and 100 μg mL^{-1}. Also compounds **5a** and **5c** exhibited excellent antimycobacterial activity (% inhibition 94 ± 0.01, 96.54 ± 0.90) at MIC 50 μg mL^{-1} and 62.5 μg mL^{-1} against *M.tuberculosis* $H_{37}RV$. 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^{-1} against *M.tuberculosis* $H_{37}RV$. Compounds **5f**, **5i** and **5e** displayed less antimycobacterial activity (% inhibition 45 ± 0.06, 57.10 ± 0.41 and 38.24 ± 0.57) at MIC 200, μg mL^{-1} towards *M.tuberculosis* $H_{37}RV$. Compound **5g** showed no inhibition, as displayed in Table 3. The presence of the 1,3,4-oxadiazole and 1,3,4-thiadiazole ring as pharmacophores and the increase in the lipophilic character of the molecule due to the presence of substituted calix [4]arene, which facilitates the crossing through the biological membrane of the micro-organism thereby inhibiting their growth.

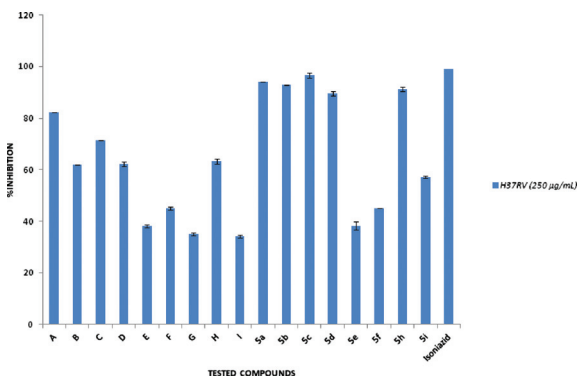


Fig. 3 Antimycobacterial activity (% inhibition) of compounds (**A–I**) and (**5a–5i**).

Antioxidant activity

Free radical scavenging activity of the tested compounds **5a–5i** was studied by the DPPH (diphenyl picryl hydrazyl) assay method.[51] The drug stock solution (1 mg mL^{-1}) was diluted to final concentrations of 2, 4, 6, 8 and 10 μg mL^{-1} 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

Table 3 *In vitro* antituberculosis activity % inhibition against *M.tuberculosis* $H_{37}Rv$ at concentration 250 μg mL^{-1} at (MIC μg mL^{-1}) 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**)

In vitro antimycobacterial activity		
Compound no	MIC μg mL^{-1} [a]	% Inhibition
A	50	82.27 ± 0.04
B	100	62.00 ± 0.02
C	100	71.50 ± 0.03
D	100	62.21 ± 0.80
E	250	38.24 ±0.57
F	100	45.00 ± 0.60
G	200	35.00 ± 0.64
H	100	63.23 ± 0.90
I	125	34.10 ± 0.51
5a	50	94.00 ± 0.01
5b	150	92.80 ± 0.07
5c	62.5	96.54 ± 0.90
5d	100	89.42 ± 0.8
5e	200	38.24 ± 1.57
5f	200	45.00 ± 0.06
5g	—	—
5h	62.5	91.23 ± 0.92
5i	200	57.10 ± 0.41
Isoniazid[b]	0.2	99.00

[a] MIC (μg mL^{-1}) = Minimum inhibitory concentration. Mean of five replicates: ± Standard deviation (−) No activity. [b] Standard.

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

Antioxidant activity		
Compound no	DPPH scavenging (%)[a]	IC_{50} μg mL^{-1}
5a	62.0	8.0 ± 0.05
5b	50.0	[c]
5c	75.0	6.45 ±0.11
5d	72.3	7.23 ±0.1
5e	54.6	9.34 ± 0.17
5f	68.4	7.42 ± 0.04
5g	48.6	[c]
5h	82.6	6.1 ± 0.04
5i	64.4	8.16 ± 0.10
Ascorbic acid[b]	92.0	5.70 ± 0.00

[a] Results are mean of three different experiments. [b] Standard mean of three replicates: ± Standard deviation. [c] Low antioxidant activity (IC_{50} > 10 μg mL^{-1})

into the percentage antioxidant activity. Methanol was used as the solvent and ascorbic acid as the standard. The trials were done in triplicate. The inhibitory concentration (IC_{50}) value, represents the concentration required to exhibit 50% antioxidant activity (Table 4). In the synthesized compounds, compounds **5h** and **5c** were found to possess maximum antioxidant activity 82.6%, 75.0% and their inhibitory concentration (IC_{50}-6.1 μg mL^{-1}, IC_{50}-6.45 μg mL^{-1}) against the standard drug ascorbic acid 92% (IC_{50}-5.7 μg mL^{-1}). However compound **5f** showed moderate antioxidant activity 68.4% (IC_{50}-7.42 μg mL^{-1}), and **5b** and 5g showed minimum antioxidant activity

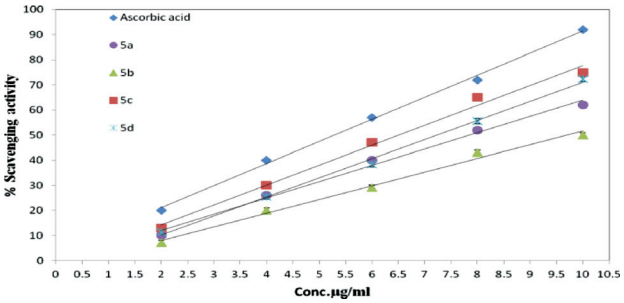


Fig. 4 Antioxidant activity of compounds **5a–d**.

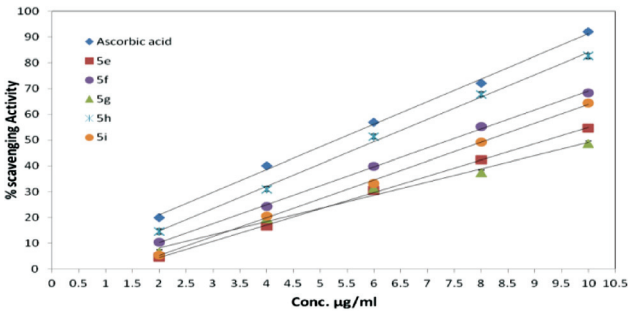


Fig. 5 Antioxidant activity of compounds **5e–i**.

(IC_{50} >10 μg mL^{-1}). The results revealed that the compounds with an oxadiazole moiety exhibited good antioxidant properties, compared to those having a thiadiazole moiety. The results are presented in Fig. 4 and Fig. 5.

Conclusion

We have synthesized a novel calix[4]arene assembly incorporating isoniazid derivatives with 1,3,4-thiadiazole and 1,3,4-oxadiazole heterocycles. *In vitro* antibacterial, antifungal and antitubercular activities showed that all compounds were efficient on two gram negative (*E. coli*, *P.aeruginosa*), two gram positive (*S. aureus*, *S. pyogenes*) reference strains, two fungi species (*C. albicans*, *A. clavatus*) and $H_{37}Rv$ bacteria compared to standard drugs. Compounds **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]areneand, and **5b**, 5,11,17,23-tetra-*tert*-butyl-25,27-bis(5-(pyridin-4-yl)-1,3,4-thiadiazole-2-amidemethyleneoxy)-26,28-dihydroxycalix[4]arene, exhibited even higher bioactivity, especially towards *M. tuberculosis*, than the 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 antimycobacterial activity. The enhancement of activity of oxadiazole and thiadiazole functionalized calix[4]arenes compared to the unsubstituted oxadiazole and thiadiazole (**A–I**) is attributed to the cooperative effect of the pharmacophores. Furthermore, compounds having an

oxadiazole moiety showed good antioxidant activity. Further, optimization and pharmacokinetic characterization of this series are in progress in our laboratory.

Materials and Method

All the chemicals and reagents were of analytical grade of BDH, Aldrich and Merck unless and otherwise specified. The solvents used for the analysis were purified by standard methods.[52] 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. Elemental analyses system used was GmbH Vario Micro cube elementar analyzer. ^{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-d_6 with tetra methyl silane (TMS) as an internal standard.

Synthesis of Nicotinic acid, benzoic acid and *cis*-cinnamic acid hydrazide

Nicotinic acid, benzoic acid and *cis*-cinnamic acid hydrazide were prepared as reported in the earlier paper.[53,54]

Synthesis of 1,3,4-oxadiazole and 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]

General procedure for the synthesis of compounds A, D, F and H

Amixture of various acid hydrazides (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 dilute 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** and **H**). The compounds (**A**, **D**, **F** and **H**) were synthesized in an analogous manner and were characterized as shown below.

5-(pyridin-4-yl)-1,3,4-oxadiazole-2-thiol (A)

Yield: 74%, mp 166 °C; IR (KBr, υ cm^{-1}): 1638 (C═N), 1521 (C═C aromatic), 1430 (C–O–C oxadiazole), 1164 (SH); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.94, 8.74 (m, 4H, Py), 13.03 (s, 1H, SH); MS: *m*/*z* 179 (M+); Anal. Calcd for $C_7H_5N_3OS$ (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%.

5-(pyridin-3-yl)-1,3,4-oxadiazole-2-thiol (D)

Yield: 74%, mp 231 °C; IR (KBr, υ cm^{-1}): 1640 (C═N), 1510 (C═C aromatic), 1420 (C–O–C oxadiazole), 1167 (SH); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.90, 8.72 (m, 4H, Py), 13.02 (s, 1H, SH); MS: *m*/*z* 179 (M+); Anal. Calcd for $C_7H_5N_3OS$ (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%.

5-(phenyl)-1,3,4-oxadiazole-2-thiol (F)

Yield: 74%, mp 219 °C; IR (KBr, υ cm^{-1}): 2940 (C–H), 1521 (C═C aromatic), 1425 (C–O–C oxadiazole), 1160 (SH); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.94, 8.74 (m, 4H, Phenyl), 12.92 (s, 1H, SH); MS: *m*/*z* 178 (M+); Anal. Calcd for $C_8H_6N_2OS$ (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%.

cis-5-(styryl)-1,3,4-oxadiazole-2-thiol (H)

Yield: 74%, mp 167 °C; IR (KBr, υ cm^{-1}): 2980 (C–H), 1515 (C═C aromatic), 1430 (C–O–C oxadiazole), 1161 (SH); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.94, 8.74 (m, 4H, Phenyl), 6.95–6.95 (dd, 2H, Styryl), 13.00 (s, 1H, SH); MS: *m*/*z* 204 (M+); Anal. Calcd for $C_{10}H_8N_2OS$ (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%.

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

A mixture of various hydrazides (0.01 mol) was dissolved in a minimum amount of 1 N HCl and ammonium thiocyanate (0.02 mol) was added afterward. The reaction mixture was heated under reflux for 8–10 h. After cooling, the product was filtered, washed with water and recrystallized from absolute alcohol to give the assorted thiosemicarbezides.

To a mixture of various thiosemicarbezides (0.01 mol) was dissolved 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 the 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**, and **I**).

The compounds (**B**, **E**, **G**, and **I**) were synthesized in an analogous manner and were characterized as shown below.

2-Amino-5-(4′-pyridyl)-1,3,4-thiadiazole (B)

Yield 70%, m.p. 240 °C; IR (KBr, υ cm^{-1}): 3340 (N–H str.), 1621–1433 (C═N), 660 (C–S–C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.50 (d, 2H, Ar-H, pyridine), 6.51 (s, 2H, NH_2); MS: *m*/*z* 178 (M+); Anal. Calcd. for $C_7H_6N_4S$ (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%.

2-Amino-5-(3′-pyridyl)-1,3,4-thiadiazole (E)

Yield 70%, m.p. 235 °C; IR (KBr, υ cm^{-1}): 3344 (N–H str.), 1625–1430 (C═N), 664 (C–S–C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.48 (d, 2H, Ar-H, pyridine), 6.45 (s, 2H, NH_2); MS: *m/z* 178 (M+); Anal. Calcd. for $C_7H_6N_4S$ (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%.

2-Amino-5-(phenyl)-1,3,4-thiadiazole (G)

Yield 70%, m.p. 223 °C; IR (KBr, υ cm^{-1}): 3342 (N–H str.), 1620–1438 (C═N), 656 (C–S–C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.46 (d, 2H, Ar-H), 6.40 (s, 2H, NH_2); MS: *m/z* 177 (M+); Anal. Calcd. for $C_8H_6N_3S$ (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%.

***cis*-2-Amino-5-(styryl)-1,3,4-thiadiazole (I)**

Yield 70%, m.p. 165 °C; IR (KBr, υ cm^{-1}): 3342 (N–H str.), 2980 (C–H str.), 656 (C–S–C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.46 (d, 2H, Ar-H), 6.40 (s, 2H, NH_2); MS: *m/z* 203 (M+); Anal. Calcd. for $C_{10}H_9N_3S$ (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%.

2-Amino-5-(pyridin-4-yl)-1,3,4-oxadiazole (C)

The mixture of isoniazid (1.37 g, 0.01 mol), the 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 and neutralized with a sodium bicarbonate solution. The solid thus precipitated was washed, dried and recrystallized from ethanol.

Yield 77%; mp 240 °C (dec); IR (KBr, υ cm^{-1}): 3340 (str NH), 1621–14330 (C═N), 1210 (C–O–C), ^{1}H NMR (DMSO-d_6) δ 8.50 (m, 4H pyridine), 6.51 (s, 2H, NH_2); ^{13}C NMR (DMSO-d_6) δ 169.2–153 (C-2 and C-5 of oxadiazole): 149.8, 124.2, 147 (C-4 of pyridine); MS: *m/z* 178 (M+); Anal. Calcd for $C_8H_{10}N_4O_4$ (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%.

Synthesis of compounds 1, 2, 3 and 4

Compounds **1**, **2**, **3** and **4** were prepared as reported in the earlier paper.[58]

General method for the synthesis of (5a–5i)

Compound **4**, 5,11,17,23-tetra-*tert*-butyl-25,27-bis(chloro-carbonyl-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 made 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 room temperature at 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 diluted with 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.

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; IR (KBr, υ cm^{-1}), 3400 (-OH), 3080 (-Ar CH), 1710 (–C═O), 1731, 1025 (C–O–C linkage), 1598, 1434, 1366 (oxadiazole ring str);^{1}H NMR (400 MHz, DMSO-d_6) δ 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_2), 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) δ 31.2, 33.2 34.5, 32.2 (Me_3C 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 (OCH_2), 120.3,137.4,150.2 (C1–C5 of pyridine ring), 164.5–167.5 (C6–C7 0f oxadiazole ring); FAB-MS (*m/z*) 1088 (M+1); Anal. Calcd for $C_{62}H_{66}N_6O_8S_2$ (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%.

5,11,17,23-Tetra-*tert*-butyl-25,27-bis(5-(pyridin-4-yl)-1,3,4-thiadiazole-2-amidemethyleneoxy)-26,28-dihydroxycalix[4]arene (5b)

Yield 82%: mp > 250 °C; IR (KBr, υ cm^{-1}): 3412 (-OH), 2989 (-Ar-CH), 1670–1656 (NHCO), 696 (C–S–C linkage), 1240 (N–N═C thiadiazole ring str);^{1}H NMR (400 MHz, DMSO-d_6), δ 0.94 (s, 18H, *tert*-butyl), 1.10 (s, 18H, *tert*-butyl), 3.80 (d, 4H, J = 12.8 Hz, Ar-CH_2-Ar), 4.10 (d, 4H, J = 12.8 Hz, Ar-CH_2-Ar), 4.39 (s, 4H, CH_2O), 6.70–7.15 (m, 8H, ArH), 8.05 (s, 2H, OH), 7.35 (s, 8H, pyH), 10.20 (d, 2H, NH), ^{13}C NMR (125 MHz, DMSO-d_6) δ 31.4, 32.9, 34.2, 33.4 (Me_3C), 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), 63.3 (OCH_2),121.3, 137.2, 149.4 (C1–C5 of pyridine ring), 163.5–166.1 (C6–C7 0f thiadiazole ring); FAB-MS (*m/z*) 1086.(M+1); Anal. Calcd for $C_{62}H_{68}N_8O_6S_2$ (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%.

5,11,17,23-Tetra-*tert*-butyl-25,27-bis(5-(pyridin-4-yl)-1,3,4-oxadiazole-2-amidemethyleneoxy)-26,28-dihydroxycalix[4]arene (5c)

Yield 85%; mp >250 °C(dec); IR (KBr, υ cm^{-1}): 3410 (-OH), 2990 (-Ar-CH), 1670–1654 (NHCO), 1210, 1025 (C–O–C linkage), 1598, 1434, 1366 (oxadiazole ring str);^{1}H NMR (400 MHz, DMSO-d_6) δ 0.96 (s, 18H, *tert*-butyl)1.15 (s, 18H, *tert*-butyl), 3.85 (d, 4H, J = 12.8 Hz, Ar-CH_2-Ar), 4.15 (d, 4H, J = 12.8 Hz, Ar-CH_2-Ar), 5.18 (s, 4H, CH_2O), 6.70–7.10 (m, 8H, ArH), 8.20 (s, 2H, OH), 7.50 (s, 8H, pyH), 10.25 (d, 2H, NH), ^{13}C NMR (125 MHz, DMSO-d_6) δ 31.43, 32.2, 34.2, 34.30 (Me_3C), 33.5 (Ar-CH_2-Ar), 169.34 (NHCO of amide), 64.4 (OCH_2), 125.4, 126.2, 127.9, 133.2, 141.7, 147.3, 148.4, 151.5

(CH of Ar) 120, 137.2, 148.5 (C1–C5 of the pyridine ring), 153,169.4 (C6–C7 of an oxadiazole ring); FAB-MS (*m*/*z*) 1054 (M+1), Anal. Calcd for $C_{62}H_{68}N_8O_8$ (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%.

5,11,17,23-Tetra-*tert*-butyl-25,27-bis(5-(pyridin-3-yl)-1,3,4-oxadiazole-2-thiacarbonylmethoxy)-26,28-dihydroxycalix[4] arene (5d)

Yield 81%: mp > 250 °C; IR (KBr, υ cm^{-1}), 3410 (-OH), 3030 (-Ar-CH), 1710 (–C═O), 1731, 1015 (C–O–C linkage), 1600, 1424, 1356 (oxadiazole ring str),^{1}H NMR (500 MHz, DMSO-d_6) δ 0.94 (s, 18H, *tert*-butyl), 1.10 (s, 18H, *tert*-butyl), 3.76 (d, 4H, J = 12.8 Hz, Ar-CH_2-Ar), 4.35 (d, 4H, J = 12.8 Hz, Ar-CH_2-Ar), 4.14 (s, 4H, OCH_2), 6.59–7.10 (m, 8H, ArH), 8.10 (s, 2H, OH), 7.20–9.10 (s, 8H, PyH), ^{13}C NMR (125 MHz, DMSO-d_6) δ 31.2, 33.20, 34.5, 32.2 (Me_3C 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 0f oxadiazole ring); FAB-MS (*m*/*z*) 1088 (M+1); Anal. Calcd for $C_{62}H_{66}N_6$ O_8S_2 (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%.

5,11,17,23-Tetra-*tert*-butyl-25,27-bis(5-(pyridin-3-yl)-1,3,4-thiadiazole-2-amidemethyleneoxy)-26,28-dihydroxycalix[4] arene. (5e)

Yield 80%: mp > 250 °C; IR (KBr, υ 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), δ 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_2O), 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) δ 31.4, 32.9, 34.2, 33.4 (Me_3C), 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 0f thiadiazole ring); FAB-MS (*m*/*z*) 1086.(M+1); Anal. Calcd for $C_{62}H_{68}N_8$ O_6S_2 (1085.38): C, 68.61; H, 6.31; N,10.32; O, 8.84; S, 5.91. Found: C, 68.58; H, 6.30; N, 10.32; O, 8.85; S, 5.88%.

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; IR (KBr, υ 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) δ 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) δ 31.3, 33.2, 34.5, 32.3 (Me_3C 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 0f oxadiazole ring); FAB-MS (*m*/*z*) 1086 (M+1); Anal. Calcd for $C_{62}H_{66}N_6$ O_8S_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, 5.14; O, 11.89; S, 5.92%.

5,11,17,23-Tetra-*tert*-butyl-25,27-bis(5-(phenyl)-1,3,4-thiadiazole-2-amidemethyleneoxy)-26,28-dihydroxycalix[4] arene (5g)

Yield 80%: mp > 250 °C; IR (KBr, υ 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), δ 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_2O), 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_3C), 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 the pyridine), 152.5–166.1 (C6–C7 0f thiadiazole ring); FAB-MS (*m*/*z*) 1084 (M+1); Anal. Calcd for $C_{62}H_{68}N_8$ O_6S_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%.

5,11,17,23-Tetra-*tert*-butyl-25,27-bis(5-(styryl)-1,3,4-oxadiazole-2-thiacarbonylmethoxy)-26,28-dihydroxycalix[4]arene (5h)

Yield 83%: mp > 250 °C; IR (KBr, υ 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, OCH2), 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, 33.2, 34.5, 32.3 (Me_3C of *tert-butyl*), 33.8 (Ar-CH_2-Ar), 121.2, 125, 126.4, 133.2, 141.7, 147.3, 150.2 (CH of Ar), 161.5 (SCO), 65.4 (OCH_2), 123.3–158.2 (C1–C5 of the pyridine ring), 161.5–169 (C6–C7 0f oxadiazole ring); FAB-MS (*m*/*z*) 1138 (M+1); Anal. Calcd for $C_{62}H_{66}N_6$ O_8S_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%.

5,11,17,23-Tetra-*tert*-butyl-25,27-bis(5-(styryl)-1,3,4-thiadiazole-2-amidemethyleneoxy)-26,28-dihydroxycalix[4]arene (5i)

Yield 82%: mp > 250 °C; IR (KBr, υ 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_3C), 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 0f thiadiazole ring); FAB-MS (*m*/*z*) 1136 (M+1); Anal. Calcd for

$C_{62}H_{68}N_8O_6S_2$ (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%.

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