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Biological Evaluation of 2, 5-Di (4 Aryloylaryloxy Methyl) - 1, 3, 4-Oxadiazoles Derivatives as Antimicrobial Agents

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Abstract

A series of potential biologically active substituted 2,5-di(4 aryloylaryloxymethyl)-1,3,4-oxadiazoles **9a-j** were evaluated for its potential antimicrobial activity comparing with the standard drugs-Streptomycin and Ketoconazole respectively. Compound **9a** with fluoro group exhibited highest activity against both gram-positive and gram-negative bacteria. Compounds **9a** with fluoro group and **9c** with fluoro and bromo showed good activity against antifungal activities.

Keywords: 1,3,4-Oxadiazoles; Synthesis; Antibacterial; Antimicrobial

Introduction

Infection of microbes is a serious problem in modern medicine. Among the most purchased drugs, antimicrobials drugs are usually used worldwide. Such a necessary treatment is needed especially in the upcoming world where infectious diseases are a common cause of death. An alarming level has been reached by the new emerging drug resistant micro-organisms around the world causing life-threatening infectious diseases. Recently, the wound infections, blood stream infections are caused by the Staphylococcus aureus and that of Diarrhoea ("bacillary dysenteria") by the Shigella species [1]. An increasing number of immuno-compromised patients as a result of HIV infection, cancer chemotherapy and organ transplantation is also one of the major factors contributing to the increasing use of antimicrobial drugs. Also, the smart arising claim for the material protection from microbial infection has paved the way for the pharmacological research [2,3]. The above-mentioned fact is the cause for a great concern creating a insistent need for new anti-microbial agents. Despite of great effort from the pharmaceutical industry to manage the resistance problem, the discovery and development of new mechanistic classes of antibiotics has found very little success [4]. The difficulty of this task has been demonstrated by the fact that only two antibiotics of new classes, linezolid (an oxazolidinone) and daptomycin (a cyclic lipopeptide), have been successfully developed in the past three decades [5,6]. In the past 20 years, the incidence of microbial infection has reached a peak level over the world as a result of resistance against the drugs. The health problems pose to explore and synthesize a novel class of antimicrobial species effective against pathogenic microorganisms that has developed resistance to the antibiotics in the current regimen [3,7]. However, additional mutations may compensate for this fitness cost and aids the survival of these bacteria. Hence, the search for a new and potent antimicrobial agents is gaining interest. When the era of synthetic drugs began, it opened up thousand doors for the development of various synthetic molecules with a potential action. The compounds with the backbone of benzophenones have been reported to possess various biological activities such as anticancer [8] antimicrobial [9] antioxidant [10]. 1,3,4-Oxadiazole ring is associated with many types of biological properties such as anti-inflammatory [11-13], hypoglycemic [14], antifungal and antibacterial [15-19] activities. 1,3,4-Oxadiazoles and its derivatives have a broad range of biological and pharmacological properties and are widely used as starting materials for the synthesis of a broad range of heterocyclic compounds and substrates for the drug synthesis. Some of its derivatives show a wide range of biological and pharmacological activity, such as anticancer [8,20] antiviral activities [21]. Prompted by these, the present paper emphasizes on the synthesis, characterization and antimicrobial evaluation of 2,5-di(4 aryloylaryloxy methyl)- 1,3,4-oxadiazoles derivatives. All the synthesized compounds were characterized on the basis of their physical properties IR, ^{1}H and ^{13}C NMR spectral data and elemental analysis. The physical data of titled compounds are summarized and present in the result and discussion part.

Materials and Methods

Experimental section

All solvents and reagents were purchased from Sigma Aldrich Chemicals Pvt Ltd. TLC was performed on aluminum-backed silica plates and visualized by UV-light. Melting points (M.P) were determined on an electrically heated VMP-III melting point apparatus. The elemental analysis of the compounds was performed on a Perkin Elmer 2400 elemental analyzer. The results of elemental analyses were within ± 0.4% of the theoretical values. The FT-IR spectra were recorded using KBr discs and Nujol on FT-IR Jasco 4100 infrared spectrophotometer. ^{1}H NMR spectra were recorded on a Bruker 400 MHz NMR spectrometer in $CDCl_3$ or DMSO and the chemical shifts were recorded in parts per million downfi ld from tetramethylsilane. Mass spectra were recorded on LC-MS (API-4000) mass spectrometer. MTT was purchased from Sigma Aldrich, USA and CD31 antibodies were procured from Santa Cruz, USA.

Synthesis

General procedure for substituted arylbenzoates (3a-e): 2-Chloro-6-fluoro phenol (1, 0.2054 mol) was dissolved in DCM, triethylamine (TEA, 0.4519 mol) was added and the reaction mixture was cooled to 0°C. A solution of benzoyl chloride derivatives (**2a-e**, 0.2157 mol) in DCM was added slowly to the above mixture and

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stirred for 3 h. Then the reaction mass was diluted with DCM (200 mL), washed with 10% sodium hydroxide solution (3 × 30 mL), water (3 × 30 mL), brine (2 × 60 mL), and again with water (3 × 30 mL). The organic layer was dried over sodium sulfate and the solvent was evaporated to achieve compounds **3a-e** [8].

Synthesis of 2-chloro-6-fluorophenyl-4-fluorobenzoate (3a): 2-Chloro-6-fluoro phenol (1, 30 g, 0.2054 mol) was dissolved in DCM, triethylamine (TEA, 45.73 g, 0.4519 mol) was added and the reaction mixture was cooled to 0°C. A solution of 4-fluorobenzoyl chloride (**2a**, 33.9 g, 0.2157 mol) in DCM was added slowly to the above mixture and internal temperature was maintained to 0-10°C. Finally the reaction mixture was stirred at ambient temperature for 3 h. Then the reaction mass was diluted with DCM (200 mL), washed with 10% sodium hydroxide solution (3 × 30 mL), water (3 × 30 mL), brine (2 × 60 mL), and again with water (3 × 30 mL). The organic layer was dried over sodium sulfate and the solvent was evaporated to achieve compound 3a as white solid. Yield: 94%; m.p.: 52.6-54.1°C; IR (KBr) nmax (cm^{-1}): 1738 (ester, C=O); ^{1}H NMR (400 MHz) (DMSO-d6) d (ppm): 7.42-7.53 (m, 4H, Ar-H), 8.25-8.28 (m, 3H, Are-H); MS (EI): m/z (75%) M+1 268.5; Anal. Calcd. for $C_{13}H_7ClF_2O_2$ (268.5): C, 58.12; H, 2.63; Cl, 13.20; F, 14.14. Found: C, 58.22; H, 2.43; Cl, 13.30; F, 14.29%. Compounds 3b-e were synthesized analogously starting with 2b-e respectively by same method [8].

General procedure for (4-hydroxyaryl)aryl methanones (4a-e): Compound **3a-e** (0.1903 mol) and aluminum chloride (0.5388 mol) were blended and the mixture was heated to 150°C and this temperature was maintained for 1 h. Then the reaction mixture was cooled to 0°C and quenched with 6 N hydrochloric acid (200 mL) and extracted with DCM (3 × 100 mL). The organic layer was washed with water (3 × 40 mL), brine (3 × 30 mL) and again with water (3 × 40 mL). The organic layer was dried over anhydrous sodium sulfate and the solvent was evaporated to afford compounds **4a-e**.

Synthesis of (3-chloro-5-fluoro-4-hydroxyphenyl)4- fluorophenyl methanone (4a): Compound 3a (51 g, 0.1903 mol) and aluminum chloride (71.05 g, 0.5388 mol) were blended and the mixture was heated to 150°C and this temperature was maintained for 1 h. Then the reaction mixture was cooled to 0°C and quenched with 6 N hydrochloric acid (200 mL) and extracted with DCM (3 × 100 mL). The combined organic layer was washed with water (3 × 40 mL), brine (3 × 30 mL) and again with water (3 × 40 mL). The organic layer was dried over anhydrous sodium sulfate and the solvent was evaporated to afford compound **4a** as pale yellow solid. Yield: 61%; m.p.: 146.3-147.7°C; IR (KBr) nmax (cm^{-1}): 1671 (C=O), 3545-3635 (OH); ^{1}H NMR (400 MHz) (DMSO-d6) d (ppm): 7.36-7.82 (m, 6H, Ar-H), 11.64 (bs, 1H, OH). MS (EI): m/z (83%): M+ 268.5; Anal. Calcd. for $C_{13}H_7ClF_2O_2$ (268.5): C, 58.12; H, 2.63; Cl, 13.20; F, 14.14. Found: C, 58.21; H, 2.52; Cl, 13.20; F, 14.25%. Compounds **4b-e** were synthesized analogously starting with **3b-e** respectively by same method [8].

General procedure for ethyl 4-aryloylaryloxyacetates (5a-e): To a solution of compounds **4a-e** (0.1156 mol) in dry DMF (175 mL), potassium carbonate (0.3468 mol) and ethyl bromoacetate (0.1273 mol) were added and the reaction mass was heated to 60°C for 3 h. The reaction mass was diluted with ethyl acetate (200 mL), potassium carbonate was filtered off and the bed was washed with ethyl acetate (100 mL). The organic layer was washed with water (3 × 30 mL), brine (2 × 40 mL), dried over sodium sulfate and concentrated to yield compounds **5a-e**.

Synthesis of ethyl [2-(4-fluorobenzoyl)-2-chloro-6- fluorophenoxy] acetate (5a): To a solution of compound **4a** (31 g, 0.1156 mol) in dry DMF (175 mL), potassium carbonate (47.83 g, 0.3468 mol) and ethyl bromoacetate (21.11 g, 0.1273 mol) were added and the reaction mass was heated to 60°C and maintained for 3 h. The reaction mass was diluted with ethyl acetate (200 mL), potassium carbonate was filtered off and the bed was washed with ethyl acetate (100 mL). The organic layer was washed with water (3 × 30 mL), brine (2 × 40 mL), dried over sodium sulfate and concentrated to yield compound **5a** as brown pasty mass. Yield: 97%; IR (KBr) nmax (cm^{-1}): 1660 (C=O), 1730 (ester, C=O); ^{1}H NMR (400 MHz) (DMSO-d6) d (ppm): 1.16-1.22 (t, 3H, CH3), 4.14-4.21 (q, 2H, CH2), 5.02 (s, 2H, OCH2), 7.64-7.8 (m, 6H, Ar-H). MS (EI): m/z (59%): M+1 354.5; Anal. Calcd. for $C_{17}H_{13}ClF_2O_4$ (354.5): C, 57.56; H, 3.69; Cl, 9.99; F, 10.71. Found: C, 57.41; H, 3.52; Cl, 9.79; F, 10.88%. Compounds **5b-e** were synthesized analogously starting with **4b-e** respectively by same method [8].

General procedure for 4-aryloylaryloxyethanoic acids (6a-e): A mixture of compounds **5a-e** (0.0532 mol), 10% aqueous sodium hydroxide solution (100 mL) and THF (100 mL) was stirred at room temperature for 1 h. The reaction mass was acidifi d with 6 N hydrochloric acid (150 mL) and the aqueous layer was extracted with ethyl acetate (3 ×100 mL). The organic layer was washed with brine (3 × 60 mL), dried over anhydrous sodium sulfate and concentrated to achieve compounds **6a-e**.

Synthesis of [4-(4-fluorobenzoyl)-2-chloro-6-fluorophenoxy] ethanoic acid (6a): A mixture of compound **5a** (18 g, 0.0532 mol), 10% aqueous sodium hydroxide solution (100 mL) and THF (100 mL) was stirred at room temperature for 1 h. The reaction mass was acidifi d with 6 N hydrochloric acid (150 mL) and the aqueous layer was extracted with ethyl acetate (3 ×100 mL). The organic layer was washed with brine (3 × 60 mL), dried over anhydrous sodium sulfate and concentrated to achieve compound **6a** as white solid. Yield: 92%; m.p.: 127.3-128.6°C; IR (KBr) nmax (cm^{-1}): 1660 (C=O), 1738 (acid C=O), 3470-3575 (acid OH); ^{1}H NMR (400 MHz) (DMSO-d6) d (ppm): 4.9 (s, 2H, OCH2), 7.3-7.87 (m, 6H, AreH), 13.1 (s, 1H, COOH). MS (EI): m/z (55%): Mþ 326.5; Anal. Calcd. for $C_{15}H_9ClF_2O_4$ (326.5): C, 55.15; H, 2.78; Cl, 10.85; F, 11.63. Found: C, 55.25; H, 2.61; Cl, 10.72; F, 11.49%. Compounds **6b-e** were synthesized analogously starting with **5b-e** respectively by same method [8].

General procedure for 4-aryloylaryloxyacethydrazides (7a-e): Hydrazine hydrate (0.3372 mol) was added to a solution of compounds **6a-e** (0.0562 mol) in ethanol (100 mL) at 0ºC and stirred the reaction mixture at the same temperature for 1 h. A white solid was separated out, which was quenched with water (100 mL), filtered and washed with water (50 mL). Finally, solid was dried under vacuum to obtain compounds **7a-e**.

Synthesis of 2-[4-(4-fluorobenzoyl)-2-chloro-6-fluorophenoxy] acethydrazide (7a): Hydrazine hydrate (16.90 g, 0.3372 mol) was added to a solution of compound **6a** (19 g, 0.0562 mol) in ethanol (100 mL) at 0°C and stirred the reaction mixture at the same temperature for 1 h. A white solid was separated out, which was quenched with water (100 mL), filtered and washed with water (50 mL). Finally, solid was dried under vacuum to obtain compound **7a** as white needle.

Yield: 79%; m.p.: 107.5-109.1°C; IR (KBr) nmax (cm^{-1}): 1610 (C=O), 1645 (amide, C=O), 3100-3205 (NH-NH2); ^{1}H NMR (400 MHz) (DMSO-d6) d (ppm): 4.35 (bs, 2H, NH2), 4.69 (s, 2H, OCH2), 7.2-7.86 (m, 6H, Ar-H), 9.32 (bs, 1H, CONH). MS (EI): m/z (42%): M+ 340.5; Anal Calcd. for $C_{15}H_{11}ClF_2N_2O_3$ (340.5): C, 52.88; H, 3.25; Cl, 10.41; F, 11.15; N, 8.22. Found: C, 52.75; H, 3.38; Cl, 10.29; F, 11.24; N, 8.11%. Compounds **7b-e** were synthesized analogously starting with **6b-e** respectively by same method [8].

General procedure for N,N-di(2-(4-aryloylaryloxy)acetyl) hydrazines (8a-j): To a solution of compounds **6a-e** (0.0032 mol) in DCM (20 mL), 2,6-dimethylpyridine (0.0107 mol) and TBTU (0.00323 mol) were added at room temperature. Finally, compounds **7a-e** (0.00294 mol) were added to the reaction mixture and stirred at room temperature for 12 h. The reaction mixture was quenched with 10% sodium bicarbonate solution (20 mL) and stirred for 30 min. The solid precipitate was filtered, washed with water (20 mL) and dried to yield compounds **8a-j**.

***Synthesis of N,N-di[di(2-chloro-6-fluoro-4-(4-fluoro-benzoyl) phenoxy)]acetyl hydrazide (8a)*:** To a solution of compound **6a** (1.05 g, 0.0032 mol) in DCM (20 mL), 2,6-dimethylpyridine (1 g, 0.0107 mol) and TBTU (1.04 g, 0.00323 mol) were added at room temperature. Finally, compound **7a** (1 g, 0.00294 mol) was added to the reaction mixture and stirred at room temperature for 12 h. The reaction mixture was quenched with 10% sodium bicarbonate solution (20 mL) and stirred for 30 min. The solid precipitate was filtered, washed with water (20 mL) and dried to yield compound **8a** as white solid. Yield: 81%; m.p.: 194.8-196.2°C; IR (KBr) nmax (cm^{-1}): 1690 (C=O), 1610 (amide, C=O), 3700-3500 (NH-NH); ^{1}H NMR (400 MHz) (DMSO-d6) d (ppm): 4.90 (s, 4H, 2CH2), 7.1-7.87 (m, 12H, Ar-H), 10.36 (bs, 2H, 2NH). MS (EI): m/z (61%): M+1 649; Anal. Calcd. For $C_{30}H_{18}Cl_2F_4N_2O_6$ (649): C, 55.49; H, 2.79; N, 4.31. Found: C, 55.37; H, 2.88; N, 4.43%. Similarly compounds **8b-j** were synthesized starting from compounds **6b-e** and **7a-e** by same method [8].

General procedure for 2,5-di(4-aryloylaryloxymethyl)-1,3,4-oxadiazoles (9a-j): To a solution of compounds 8a-j (0.0023 mol) in DCM (20 mL), pyridine (0.0069 mol) and triflic anhydride (0.0051 mol) were added at 0°C and the reaction mixture was stirred at 0°C for 3 h. The reaction mass was diluted with DCM (20 mL), the organic layer was washed with 10% sodium bicarbonate (3 × 10 mL), water (3 × 10 mL) and brine (3 × 10 mL). Finally, the organic layer was dried over sodium sulfate and concentrated to yield compounds **9a-j**.

***Synthesis of 2,5-di[2-fluoro-4-(4-fluoro)benzoyl-6-chlorophenoxymethyl]1,3,4-oxadiazole (9a)*:** To a solution of compound 8a (1.5 g, 0.0023 mol) in DCM (20 mL), pyridine (0.56 g, 0.0069 mol) and triflic anhydride (1.44 g, 0.0051 mol) were added at 0°C and the reaction mixture was stirred at 0°C for 3 h. After the completion of the reaction monitored by TLC, the reaction mass was diluted with DCM (20 mL), the organic layer was washed with 10% sodium bicarbonate (3 × 10 mL), water (3 × 10 mL) and brine (3 × 10 mL). Finally, the organic layer was dried over sodium sulfate and concentrated to yield a brown gummy mass. The crude product was purifi d by column chromatography using silica gel as stationary phase and hexane: ethyl acetate as mobile phase to achieve compound **9a** as white solid. Yield; 73%; m.p.: 129.5-130.2°C; IR (Nujol) nmax (cm^{-1}): 1153 (C-O-C linkage), 1658 (C=O), 1683 cm^{-1} (C=N); ^{1}H NMR (400 MHz) (DMSO-d6) d (ppm): 5.6 (s, 4H, 2CH2), 7.05-7.81 (m, 12H, Ar-H). MS (EI): m/z (70%): M+1 631; Anal. Calcd. For $C_{30}H_{16}Cl_2F_4N_2O_5$ (631): C, 57.07; H, 2.55; N, 4.44. Found: C, 57.17; H, 2.47; N, 4.36%. Similarly compounds **9b-j** were synthesized starting from compounds **8b-j** by same method [8].

Biology

Materials and methods for the antimicrobial activity: Streptomycin was used as positive control against bacteria. Ketoconazole (Himedia, Mumbai) was used as positive control against fungi.

Tested microbes: The following gram positive bacteria were used for the experiments; *staphylococcus aureus* (MTCC 7443), *B. cereus*, *Staphylococcus aureus* (MRSA) (MTCC 84), *B. subtilis*, *Enterobacter aerogenes* (MTCC 111). The gram negative bacteria included *Escherichia coli*, *P. aeruginosa, Salmonella typhimurium* (MTCC 2488), *Klebsiella pneumoniae* (MTCC 109), and *Salmonella paratyphi-B* (MTCC 733). In addition, fungi *A. niger, Candida albicans* (MTCC 227), *Botyritis cinerea* (MTCC 2880), *F. solani*, *A. flavus*, *Candida krusei* (MTCC 231), *Malassesia pachydermatis*, *F. moniliforme, C. gloeosporioides* and *C. parapsilosis* were also used for the experiments. All cultures were obtained from the Department of Microbiology, Manasagangotri, Mysuru.

Preparation of inoculums: Bacterial inoculums were prepared by growing cells in Mueller Hinton Broth (MHA) (Himedia) for 24 h at 37°C. These cell suspensions were diluted with sterile MHB to provide initial cell counts of about 104 CFU/mL. The filamentous fungi were grown on Sabouraud Dextrose Agar (SDA) slants at 28°C for 10 days and the spores were collected using sterile double distilled water and homogenized.

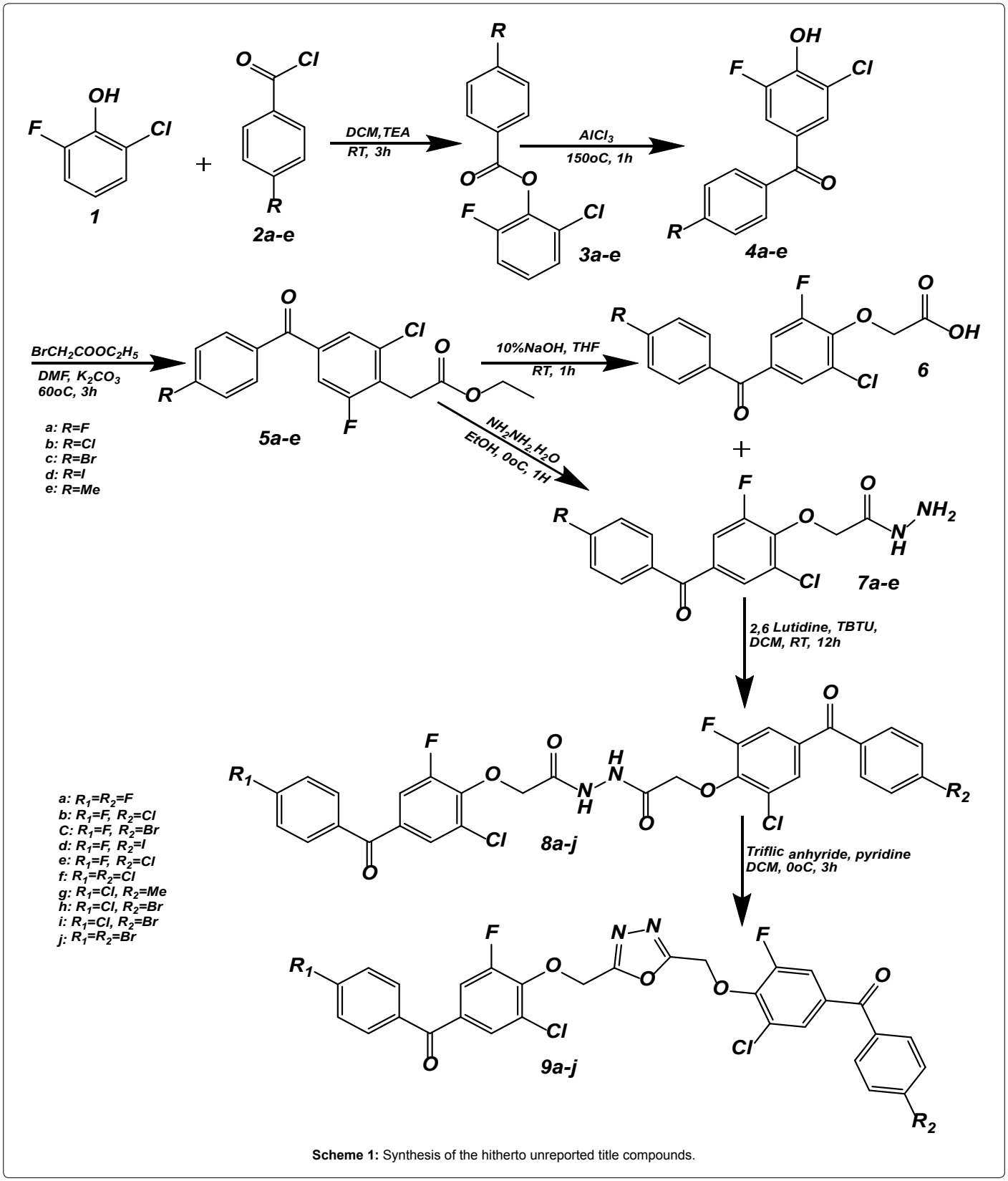
Disc diffusion assay: Antibacterial activity was carried out using a disc diffusion method (Murray) [21]. Petri plates were prepared with 20 mL of sterile Mueller Hinton Agar (MHA) (Himedia, Mumbai). The test cultures were swabbed on the top of the solidifi d media and were allowed to dry for 10 mins. The tests were conducted at 1000 µg/disc. The loaded discs were placed on the surface of the medium and left for 30 min at room temperature for compound diffusion. Negative control was prepared using respective solvent. Streptomycin (10 µg/disc) was used as positive control. The plates were incubated for 24 h at 37°C for bacteria and 48 h at 27°C for fungi. Zone of inhibition was recorded in millimeters and the experiment was repeated twice.

Minimum inhibitory concentration (MIC): Minimum inhibitory concentration studies of synthesized compounds were performed according to the standard reference method for bacteria (Duraipandiyan and Ignacimuthu) [22] and filamentous fungi (Clinical and Laboratory Standards Institute). Required concentrations (1000 µg/mL, 500 µg/mL, 250 µg/mL, 125 µg/mL, 62.5 µg/mL, 31.25 µg/mL and 15.62 µg/mL) of the compound were dissolved in DMSO (2%), and diluted to give serial two-fold dilutions that were added to each medium in 96 well plates. An inoculum of 100 mL from each well was inoculated. The anti-fungal agents ketoconazole for fungi and streptomycin for bacteria were included in the assays as positive controls. For fungi, the plates were incubated for 48-72 h at 28°C and for bacteria the plates were incubated for 24 h at 37°C. The MIC for fungi was defi ed as the lowest extract concentration, showing no visible fungal growth after incubation time. 5 Ml of tested broth was placed on the sterile MHA plates for bacteria and incubated at respective temperatures. The MIC for bacteria was determined as the lowest concentration of the compound inhibiting the visual growth of the test cultures on the agar plate.

Results and Discussion

Chemistry

The synthesis of the hitherto unreported title compounds is as outlined in Scheme 1. (4 Hydroxyaryl)aryl methanones commonly known as hydroxybenzophenones **4a-e** were achieved in excellent yield using benzoylation of compound **1** with benzoyl chloride derivatives **2a-e** followed by Fries rearrangement of substituted arylbenzoates **3a-e**. Compounds **4a-e** on reaction with ethyl bromoacetate afforded ethyl 4-aryloylaryloxyacetates **5a-e** which on treatment with sodium hydroxide in presence of THF gave 4- aryloylaryloxyethanoic acids **6a-e**. Further, compounds **5a-e** on treatment with hydrazine hydrate in the presence of ethanol yield 4-aryloylaryloxyacethydrazides



Scheme 1: Synthesis of the hitherto unreported title compounds.

7a-e. Condensation of **6a-e** with **7a-e** in the presence of 2,6 lutidine, O-(benzotriazol-1-yl)-N,N,N0,N0-tetramethyluronium tetrafluoroborate (TBTU) and dichloromethane (DCM) afforded N,N-di(2-(4-aryloylaryloxy) acetyl)hydrazines **8a-j**. Finally, title compounds **9a-j** were achieved by intramolecular cyclization of **8a-j** in the presence of triflic nhydride, pyridine and DCM.

In vitro anti-microbial activity

Anti-bacterial activity assay: The anti-bacterial screenings of the synthesized compounds were undertaken using disc diffusion method. The screening results of the tested compounds against the gram positive bacteria *(Staphylococcus aureus* (*MTCC 7443), B. cereus, Staphylococcus aureus (MRSA) (MTCC 84), B. subtilis, M. luteus, Enterobacter aerogenes (MTCC 111),* gram negative bacteria *(Escherichia coli, P. aeruginosa, P. vulgaris, Salmonella typhimurium (MTCC 2488), Klebsiella pneumoniae (MTCC 109), Salmonella paratyphi-B (MTCC 733),* in addition to the pathogenic fungi *A. niger,* Candida *albicans* (*MTCC 227*), *Botyritis cinereal* (*MTCC 2880*), *F. solani, A. flavus, Candida krusei* (*MTCC 231*), *Malassesia pachydermatis, F. moniliforme C. gloeosporioides C. parapsilosis* are summarized in Figure 1 and Table 1.

In antibacterial activity the obtained data revealed that most of the compounds showed moderate to excellent activities against the tested microorganisms. Among all the synthesized substituted 2,5-di(4 aryloylaryloxy methyl)- 1,3,4-oxadiazoles compounds **9a-j**, compounds **9a** with fluoro group exhibited highest activity compared with the standard drug Streptomycin. Compounds **9c** with fluoro and bromo groups has shown second highest activity. Further, compounds **9b** with fluoro and chloro groups, **9d** with fluoro and iodo groups, **9f** with chloro group and **9j** with bromo group also exhibited moderate activity.

Anti-fungi activity assay: The anti-bacterial screenings of the synthesized compounds were undertaken using disc diffusion method. The screening results of the tested compounds against the pathogenic fungi *A. niger, Candida albicans* (*MTCC 227*), *Botyritis cinereal* (*MTCC 2880*), *F. solani, A. flavus, Candida krusei* (*MTCC 231*), *Malassesia pachydermatis, F. moniliforme, C. gloeosporioides, C. parapsilosis* are summarized in Figure 2 and Table 2.

In anti-fungi activity assay the obtained data shown that most of the compounds showed moderate to excellent activities against the tested microorganisms. Among all the synthesized substituted 2,5-di(4 aryloylaryloxy methyl)- 1,3,4-oxadiazoles compounds **9a-j**, compounds **9a** with fluoro group exhibited highest activity compared with the standard drug Ketoconazole. Compounds **9b** with fl oro and chloro groups, **9c** with fluoro and bromo groups, **9d** with fl oro and iodo groups and **9h** with chloro and iodo groups has shown good activity. Further, compounds, **9i** with chloro and bromo groups and **9j** with bromo group also showed moderate activity.

Conclusion

From the results of the present study, it is concluded that, a series of novel biologically active substituted 2,5-di(4 aryloylaryloxymethyl)-1,3,4-oxadiazoles **9a-j** were synthesized and screened for antimicrobial activity and were compared with standard drugs-Streptomycin and Ketoconazole respectively. The antibacterial activity result shows that compound **9a** with fluoro group exhibited highest activity. Compounds **9c** with fluoro and bromo groups has shown second highest activity. Further, compounds **9b** with fluoro and chloro groups, **9d** with fl oro and iodo groups, **9f** with chloro group and **9j** with bromo group also exhibited moderate activity. Further, The Antifungal activity of the compounds **9a-j** result shows that compound **9a** with fl oro group exhibited highest activity. Compounds 9b with fluoro and chloro groups, **9c** with fluoro and bromo groups, **9d** with fluoro and iodo groups and **9h with** chloro and iodo groups has shown good activity.

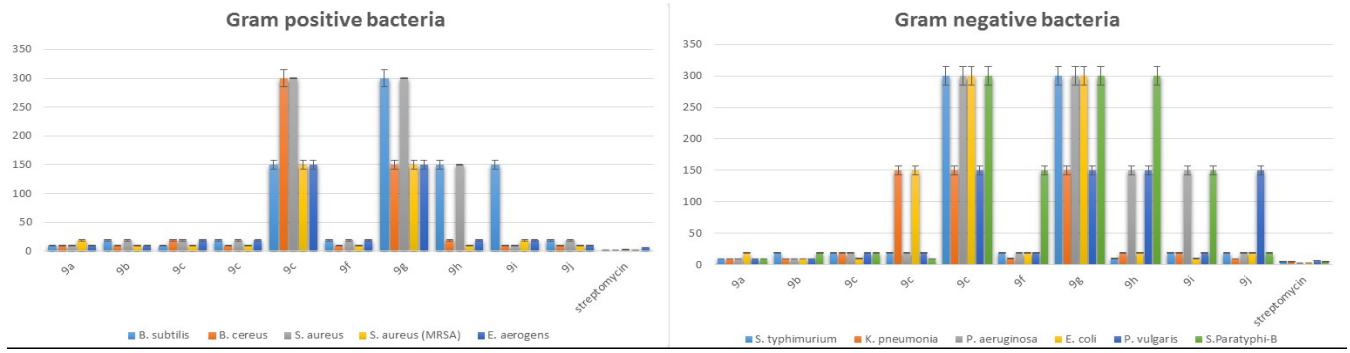


Figure 1: The inhibitory effects of compounds 9a-j on Gram positive and Gram negative bacteria.

Compounds	Name of the microorganism MIC in mg /mL									
	A. niger	*C. albicans*	*A. flavus*	*B. cinerea*	*C. krusei*	*F. solani*	*C. parapsilosis*	*M. pachydermatis*	*F. moniliforme*	*C. gloeosporioides*
9a	9.37	9.37	9. 37	18.75	9.37	9.37	9.37	18.75	9.37	9.37
9b	9.37	18.75	9.37	9.37	18.75	9.37	9.37	18.75	18.75	9.37
9c	9. 37	18.75	18.75	9. 37	18.75	9. 37	9. 37	18.75	18.75	9. 37
9d	9.37	150	18.75	9.37	9.37	18.75	18.75	9.37	150	9.37
9e	300	300	150	150	300	150	300	300	300	150
9f	18.75	150	9.37	18.75	9.37	9.37	9.37	18.75	150	18.75
9g	150	150	300	300	150	300	300	300	150	300
9h	9.73	9.73	18.75	9.73	18.75	18.75	150	9.73	150	18.75
9i	18.75	9. 37	9. 37	18.75	150	9. 37	18.75	18.75	9. 37	150
9j	18.75	9.37	9.37	18.75	9.37	18.75	18.75	18.75	9.37	150
Ketoconazole	2.4	4.6	1.2	4.7	2.33	2.34	4.68	4.68	2.34	2.34

Table 1: Antibacterial activity of the compounds: **9a-j:** MIC minimum inhibitory concentration MIC in **µg**g /mL.

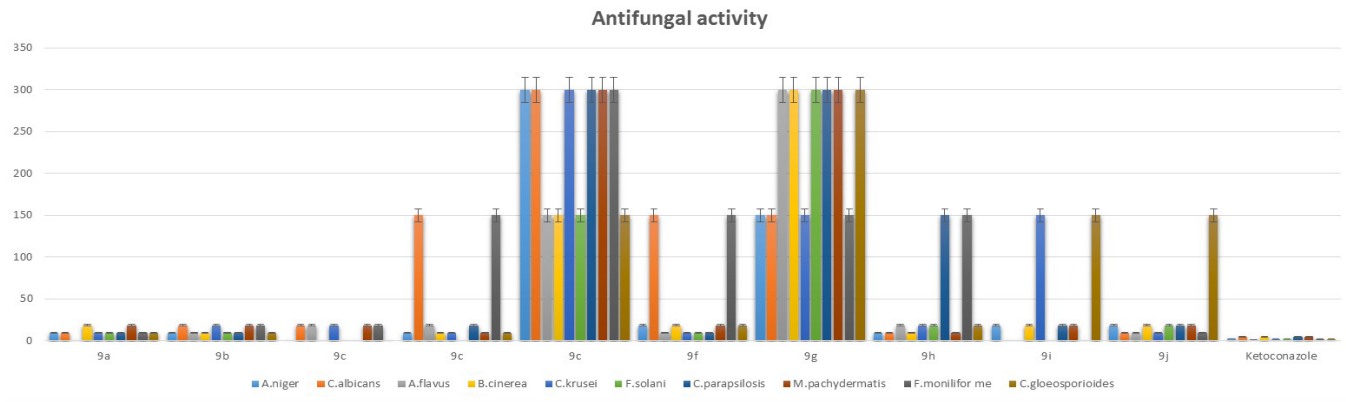


Figure 2: The inhibitory effects of compounds 9a-j on different fungal strain.

Compounds	Name of microorganism (MIC in mg g /mL)											
	Gram positive bacteria						Gram negative bacteria					
	B. subtilis	***B. cereus***	***S. aureus***	***S. aureus* (MRSA)**	***M. luteus***	***E. aerogens***	***S. typhimurium***	***K. pneumonia***	***P. aeruginosa***	***E. coli***	***P. vulgaris***	***S. Paratyphi*-B**
9a	9.37	9.37	9.37	18.75	9.37	9.37	9.37	9.37	9.37	18.75	9.37	9.37
9b	18.75	9.37	18.75	9.37	9.37	18.75	18.75	9.37	9.37	9.37	9.37	18.75
9c	9.37	18.75	18.75	9.73	18.75	9.37	18.75	18.75	18.75	9.73	18.75	18.75
9d	18.75	9.37	18.75	9.37	18.75	9.37	18.75	150	18.75	150	18.75	9.37
9e	150	300	300	150	150	300	300	150	300	300	150	300
9f	18.75	9.73	18.75	9.73	18.75	9.73	18.75	9.73	18.75	18.75	18.75	150
9g	300	150	300	150	150	300	300	150	300	300	150	300
9h	150	18.75	150	9.73	18.75	150	9.73	18.75	150	18.75	150	300
9i	150	9.73	9.73	18.75	18.75	150	18.75	18.75	150	9.73	18.75	150
9j	18.75	9.37	18.75	9.37	9.37	150	18.75	9.37	18.75	18.75	150	18.75
Streptomycin	2.34	1.17	2.35	2.38	6.34	4.5	4.68	4.68	2.34	2.34	6.68	4.68

Table 2: Antifungal activity of the compounds: **9a-j** MIC in **µg**g /Ml.

Further, compounds, **9i** with chloro and bromo groups and **9j** with bromo group also showed moderate activity.

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