

ORIGINAL ARTICLE

Activated fly ash catalyzed facile synthesis of novel spiro imidazolidine derivatives as potential antibacterial and antifungal agents

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Abstract

An array of novel spiro imidazolidine derivatives was synthesized in *dry media* and was screened for their anti-microbial activities. Structure-activity relationship results revealed that compounds 22, 23 against *P.aeruginosa*, 24 against *S.aureus*, 24, 25 against *K.pneumonia*, 27 against *S.aureus*, β -*H.streptococcus*, 29 against *M.luteus*, *K.pneumonia*, 29, 30 against *P.vulgaris* exhibited excellent antibacterial activity at a minimum inhibitory concentration (MIC) value of 6.25 μ g/mL. Compound 23 against *M.gypseum*, 25, 29 against *Candida* 6 and 29, 30 against *C.albicans* revealed excellent antifungal activity at a MIC value of 6.25 μ g/mL.

Keywords: Spiro imidazolidine derivatives, ethylene diamine, activated fly ash, antibacterial activity, antifungal activity

Introduction

Fly ash generated during the combustion of coal for energy production is one of the industrial by product and it is recognized as an environmental pollutant¹. During the initial stages, only its pozzolanic activity was paid attention². Many researchers devoted themselves to the research of the potential activity of Fly ash and the hydration process of Fly ash cement³. Activated fly ash was used to catalyze Knoevenagel condensation, 'one-pot' conversions of ketones to amides *via*, Beckmann rearrangement, Schiff Bases formation, Biginelli, and Hantzsch reactions⁴, 'one-pot' synthesis of 1,2,4,5-tetrazines⁵, microwave-assisted, solvent-free alkylation and acylation of 2-mercaptobenzothiazole⁶, different ether and ester derivatives of carvacrol⁷, liquid phase esterification of salicylic acid⁸ with acetic anhydride and methanol respectively, grindstone chemistry solid-state reaction procedure for the synthesis of heterocyclic oximes⁹, and 'one-pot' synthesis of pyrimidino thiazolidin-4-ones¹⁰. The importance of imidazolidine unit arises, because they are found in many biologically active compounds^{11,12}. In

organic synthesis, imidazolidine units are also used as synthetic intermediates^{13,14}, chiral auxiliaries¹⁵, chiral catalysis¹⁶, and ligands for asymmetric catalysis¹⁷. Like imidazole, imidazolidine-based compounds have been used as N-heterocyclic carbene ligands¹⁸ on various transition metals. It is found in the commercially available second generation Grubbs' catalyst. Many imidazolidines are biologically active. Most bio-active derivatives bear a substituent (aryl or alkyl group) on the carbon between the nitrogen centers. Oxymetazoline¹⁸ is a selective α -1 agonist and partial α -2 agonist topical decongestant. Xylometazoline is a drug which is used as a nasal decongestant. Tetrahydrozoline, a derivative of imidazolidine, is an α -agonist¹⁹ and its main mechanism of action is the constriction of conjunctival blood vessels. This serves to relieve the redness of the eye caused by minor ocular irritants. Naphazoline is a sympathomimetic agent²⁰ with marked α -adrenergic activity. Clonidine is used as a direct-acting α -2 adrenergic agonist. An imidazole ring is part of some anti-tumor drugs such as dacarbazine and imuran²¹ as well the tumor growth inhibiting

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cyclocreatine²². Recently, there has been a great deal of interest in exploiting more than one proximal functional groups for designing new structures capable of performing a variety of functions. The present study describes the use of 6-carbomethoxy-3,5-diarylcyclohex-2-enone²³ as a key intermediate with three versatile functional groups i.e., ketone, olefin, and ester for the synthesis of imidazolidine derivatives. Owing to our interest in synthesis of fascinating biologically active hybrid heterocyclic compounds^{24–26} and as part of our research program, herein we planned to design a spiro imidazolidine derivatives bearing a cyclohexene substituent on the carbon between the nitrogen centers to give a new series of spiro imidazolidine heterocycles because most bio-active imidazolidine derivatives bear a substituent (aryl or alkyl group) on the carbon between the nitrogen centers^{18–21}.

Experimental

Chemistry

General remarks

We used thin layer chromatography (TLC) to assess the progress of the reactions. All the reported melting points were taken in open capillaries and were uncorrected. IR spectra were recorded in KBr (pellet forms) on a Thermo Nicolet-Avatar-330 FT-IR spectrophotometer and noteworthy absorption values (cm⁻¹) alone were listed. One-dimensional ¹H and ¹³C NMR spectra were recorded at 400 MHz and 100 MHz, respectively on Bruker AMX 400 NMR spectrometer using DMSO-*d* as solvent. Two dimensional Heteronuclear Single-Quantum Coherence (HSQC) spectrum was recorded at 500 MHz on Bruker DRX 500 NMR spectrometer using DMSO-*d* as solvent. The electron spray impact positive (+ve) mass spectra (MS) were recorded on a Bruker Daltonics liquid chromatography-mass spectrometry spectrometer. Satisfactory microanalyses were obtained on Carlo Erba 1106 CHN analyzer. Gyan Ease Flash column chromatography system, Italy was used for flash column chromatography. BIOTAGE Initiator microwave synthesizer, Sweden a scientific microwave oven was used for the irradiation.

By adopting the literature procedure, 1,3-diaryl-prop-2-en-1-ones **1–10**²⁷ and 6-carbomethoxy-3,5-diarylcyclohex-2-enone **11–20**²³ were prepared.

General procedure for the synthesis of 7,9-diaryl-1,4-diazaspiro[4.5]-deca-9-ene-6-ethyl carboxylates **21–30**

In a 10 mL pyrex glass tube were placed respective 6-carbomethoxy-3,5-diarylcyclohex-2-enones (0.01 mol), ethylene diamine (0.01 mol) and catalytic amount of activated fly ash (15 mg). The top of glass tube was closed with teflon cover and it was placed in a teflon outer jacket and then the reaction tube was placed into the holder in the microwave cavity. The sample was irradiated under focused monomode irradiation at 90°C for 4–8 min at 2 bar pressure. After allowing the mixture to cool to room temperature, the reaction vessel was opened and the contents were poured into ice cold water. The organic material was extracted with ethyl acetate. The organic

layer was washed with 10% sodium hydrogen carbonate, brine solution and then excess of water and dried over anhydrous magnesium sulfate. After evaporation of the ethyl acetate under vacuum, a solid mass was obtained, which was subjected to flash column chromatography using toluene-ethyl acetate as eluent.

Spectroscopic data

7,9-diphenyl-1,4-diazaspiro[4.5]-deca-9-ene-6-ethyl carboxylate **21**

IR (KBr) (cm⁻¹): 3448, 3388, 3175, 3054, 3022, 2972, 2931, 2837, 1738, 1599, 1447, 1027, 827, 758, 698; ¹H NMR (δ ppm): 0.89–0.90 (*t*, 3H, ester CH₃, *J* = 7.1 Hz); 2.97–3.02 (*m*, 2H, H₈), 3.04–3.22 (*m*, 1H, H₇), 3.51–3.67 (*m*, 1H, H₆), 3.87–3.92 (*q*, 2H, ester CH₂, *J* = 6.8 Hz), 4.02–4.13 (*m*, 4H, imidazolidine 2CH₂), 5.98 (*s*, 2H, imidazolidine 2NH), 6.54 (*s*, 1H, H₁₀), 7.10–7.79 (*m*, 10H, Ar-H's); In the D₂O exchanged ¹H NMR spectrum, singlet at 5.98 ppm which resonates due to 2NH's of imidazolidine moiety disappeared; ¹³C NMR (δ ppm): 13.7 ester CH₃, 35.2 C-7, 43.8 C-8, 49.3 C-2, and C-3, 58.7 ester CH₂, 59.9 C-6, 121.9 C-10, 122.88–130.4 Ar-C's, 137.3, 141.4 *ipso*-C, 169.2 C=O.

7-(4-chlorophenyl)-9-phenyl-1,4-diazaspiro[4.5]-deca-9-ene-6-ethyl carboxylate **22**

IR (KBr) (cm⁻¹): 3535, 3437, 3382, 3295, 3065, 2978, 2924, 2847, 1735, 1601, 1445, 1018, 824, 758, 693; ¹H NMR (δ ppm): 0.92–0.93 (*t*, 3H, ester CH₃, *J* = 7.2 Hz); 2.97–3.07 (*m*, 2H, H₈), 3.10–3.14 (*m*, 1H, H₇), 3.57–3.76 (*m*, 1H, H₆), 3.89–3.94 (*q*, 2H, ester CH₂, *J* = 6.6 Hz), 4.02–4.15 (*m*, 4H, imidazolidine 2CH₂), 6.12 (*s*, 2H, imidazolidine 2NH), 6.55 (*s*, 1H, H₁₀), 7.13–7.79 (*m*, 9H, Ar-H's); In the D₂O exchanged ¹H NMR spectrum, singlet at 6.12 ppm which resonates due to 2NH's of imidazolidine moiety disappeared; ¹³C NMR (δ ppm): 13.7 ester CH₃, 34.9 C-7, 43.1 C-8, 48.4 C-2, and C-3, 58.5 ester CH₂, 60.0 C-6, 121.9 C-10, 122.88–140.4 Ar-C's, 159.1, 160.4 *ipso*-C, 169.0 C=O.

7-(4-fluorophenyl)-9-phenyl-1,4-diazaspiro[4.5]-deca-9-ene-6-ethyl carboxylate **23**

IR (KBr) (cm⁻¹): 3519, 3388, 3262, 3186, 3058, 2977, 2930, 2863, 1739, 1604, 1443, 1023, 832, 758, 694; ¹H NMR (δ ppm): 0.88–0.90 (*t*, 3H, ester CH₃, *J* = 7.0 Hz); 2.74–2.94 (*m*, 2H, H₈), 2.98–3.13 (*m*, 1H, H₇), 3.56–3.88 (*m*, 1H, H₆), 3.87–3.92 (*q*, 2H, ester CH₂, *J* = 6.8 Hz), 3.95–4.13 (*m*, 4H, imidazolidine 2CH₂), 6.02 (*s*, 2H, imidazolidine 2NH), 6.57 (*s*, 1H, H₁₀), 6.83–7.94 (*m*, 9H, Ar-H's); In the D₂O exchanged ¹H NMR spectrum, singlet at 6.02 ppm which resonates due to 2NH's of imidazolidine moiety disappeared; ¹³C NMR (δ ppm): 13.7 ester CH₃, 35.2 C-7, 42.9 C-8, 48.4 C-2, and C-3, 59.8 ester CH₂, 61.0 C-6, 122.3 C-10, 113.1–136.1 Ar-C's, 157.8, 158.2 *ipso*-C, 169.1 C=O.

7-(4-methylphenyl)-9-phenyl-1,4-diazaspiro[4.5]-deca-9-ene-6-ethyl carboxylate **24**

IR (KBr) (cm⁻¹): 3454, 3399, 3267, 3054, 3038, 2981, 2921, 2863, 1739, 1604, 1023, 832, 758, 694; ¹H NMR (δ ppm): 0.92–0.96 (*t*, 3H, ester CH₃, *J* = 6.9 Hz); 2.26 (*s*, 3H, CH₃ of phenyl ring), 2.72–2.93 (*m*, 2H, H₈), 2.97–3.12 (*m*, 1H, H₇), 3.56–3.76 (*m*, 1H, H₆), 3.87–3.93 (*q*, 2H, ester CH₂, *J* = 6.6 Hz), 4.00–4.09 (*m*, 4H, imidazolidine 2CH₂), 5.94 (*s*,

2H, imidazolidine 2NH), 6.53 (*s*, 1H, H_{10}), 6.88–7.91 (*m*, 9H, Ar-H's); In the D_2O exchanged 1H NMR spectrum, singlet at 5.94 ppm which resonates due to 2NH's of imidazolidine moiety disappeared; ^{13}C NMR (δ ppm): 13.7 ester CH_3 , 20.5 CH_3 of phenyl ring, 35.3 C-7, 43.3 C-8, 48.9 C-2, and C-3, 59.8 ester CH_2 , 60.9 C-6, 121.9 C-10, 122.8–138.4 Ar-C's, 159.2, 160.0 *ipso*-C, 169.1 C=O.

7-(4-methoxyphenyl)-9-phenyl-1,4-diazaspiro[4.5]-deca-9-ene-6-ethyl carboxylate **25**

IR (KBr) (cm^{-1}): 3524, 3424, 3267, 3054, 2989, 2929, 2830, 1739, 1445, 1607, 1033, 832, 760, 694; 1H NMR (δ ppm): 0.92–0.94 (*t*, 3H, ester CH_3 , $J=7.1$ Hz); 2.69–2.93 (*m*, 2H, H_8), 2.97–3.12 (*m*, 1H, H_7), 3.58–3.64 (*m*, 1H, H_6), 3.72 (*s*, 3H, OCH_3 of phenyl ring), 3.88–3.93 (*q*, 2H, ester CH_2 , $J=6.9$ Hz), 3.96–4.36 (*m*, 4H, imidazolidine $2CH_2$), 5.94 (*s*, 2H, imidazolidine 2NH), 6.61 (*s*, 1H, H_{10}), 6.85–7.78 (*m*, 9H, Ar-H's); In the D_2O exchanged 1H NMR spectrum, singlet at 5.94 ppm which resonates due to 2NH's of imidazolidine moiety disappeared; ^{13}C NMR (δ ppm): 13.7 ester CH_3 , 35.4 C-7, 43.0 C-8, 48.5 C-2 & C-3, 54.9 OCH_3 of phenyl ring, 59.8 ester CH_2 , 60.9 C-6, 121.9 C-10, 113.1–158.2 Ar-C's, 159.3, 160.6 *ipso*-C, 169.2 C=O.

9-(4-chlorophenyl)-7-phenyl-1,4-diazaspiro[4.5]-deca-9-ene-6-ethyl carboxylate **26**

IR (KBr) (cm^{-1}): 3524, 3386, 3175, 3059, 3029, 2978, 2927, 2858, 1738, 1448, 1584, 1013, 824, 762, 699; 1H NMR (δ ppm): 0.90–0.91 (*t*, 3H, ester CH_3 , $J=7.2$ Hz); 2.71–2.97 (*m*, 2H, H_8), 3.00–3.15 (*m*, 1H, H_7), 3.60–3.78 (*m*, 1H, H_6), 3.88–3.93 (*q*, 2H, ester CH_2 , $J=6.9$ Hz), 3.97–4.13 (*m*, 4H, imidazolidine $2CH_2$), 6.08 (*s*, 2H, imidazolidine 2NH), 6.59 (*s*, 1H, H_{10}), 6.88–7.79 (*m*, 9H, Ar-H's); In the D_2O exchanged 1H NMR spectrum, singlet at 6.08 ppm which resonates due to 2NH's of imidazolidine moiety disappeared; ^{13}C NMR (δ ppm): 14.2 ester CH_3 , 35.1 C-7, 43.0 C-8, 48.3 C-2, and C-3, 59.9 ester CH_2 , 61.0 C-6, 122.8 C-10, 114.3–137.6 Ar-C's, 141.4, 159.2 *ipso*-C, 168.9 C=O.

9-(4-methoxyphenyl)-7-phenyl-1,4-diazaspiro[4.5]-deca-9-ene-6-ethyl carboxylate **27**

IR (KBr) (cm^{-1}): 3450, 3444, 3065, 3033, 2924, 2852, 1736, 1605, 1447, 1037, 757, 695; 1H NMR (δ ppm): 0.90–0.92 (*t*, 3H, ester CH_3 , $J=7.0$ Hz); 2.72–2.98 (*m*, 2H, H_8), 3.01–3.16 (*m*, 1H, H_7), 3.59–3.71 (*m*, 1H, H_6), 3.73 (*s*, 3H, OCH_3 of phenyl ring), 3.88–3.93 (*q*, 2H, ester CH_2 , $J=6.8$ Hz), 3.96–4.08 (*m*, 4H, imidazolidine $2CH_2$), 5.93 (*s*, 2H, imidazolidine 2NH), 6.51 (*s*, 1H, H_{10}), 6.83–7.81 (*m*, 9H, Ar-H's); In the D_2O exchanged 1H NMR spectrum, singlet at 5.93 ppm which resonates due to 2NH's of imidazolidine moiety disappeared; ^{13}C NMR (δ ppm): 13.7 ester CH_3 , 36.0 C-7, 43.7 C-8, 49.2 C-2, and C-3, 54.3 OCH_3 of phenyl ring, 59.9 ester CH_2 , 61.0 C-6, 122.3 C-10, 116.2–141.4 Ar-C's, 157.8, 159.2 *ipso*-C, 169.0 C=O.

9-(4-chlorophenyl)-7-(4-methylphenyl)-1,4-diazaspiro[4.5]-deca-9-ene-6-ethyl carboxylate **28**

IR (KBr) (cm^{-1}): 3459, 3386, 3273, 3169, 3049, 2983, 2923, 2856, 1739, 1612, 1447, 1013, 817, 744, 711; 1H NMR (δ ppm): 0.92–0.94 (*t*, 3H, ester CH_3 , $J=7.1$ Hz); 2.27 (*s*, 3H, CH_3 of phenyl ring), 2.75–2.98 (*m*, 2H, H_8), 2.98–3.15 (*m*, 1H, H_7), 3.57–3.77 (*m*, 1H, H_6), 3.87–3.93 (*q*, 2H, ester

CH_2 , $J=6.9$ Hz), 4.00–4.10 (*m*, 4H, imidazolidine $2CH_2$), 6.00 (*s*, 2H, imidazolidine 2NH), 6.55 (*s*, 1H, H_{10}), 6.89–7.98 (*m*, 8H, Ar-H's); In the D_2O exchanged 1H NMR spectrum, singlet at 6.00 ppm which resonates due to 2NH's of imidazolidine moiety disappeared; ^{13}C NMR (δ ppm): 14.2 ester CH_3 , 20.5 CH_3 of phenyl ring, 35.5 C-7, 43.2 C-8, 48.8 C-2, and C-3, 59.8 ester CH_2 , 61.0 C-6, 122.2 C-10, 123.2–154.5 Ar-C's, 157.8, 159.2 *ipso*-C, 169.0 C=O.

7-(4-chlorophenyl)-9-(4-methoxyphenyl)-1,4-diazaspiro[4.5]-deca-9-ene-6-ethyl carboxylate **29**

IR (KBr) (cm^{-1}): 3442, 3393, 3284, 3065, 2962, 2923, 2850, 1738, 1598, 1457, 1140, 826, 718; 1H NMR (δ ppm): 0.92–0.94 (*t*, 3H, ester CH_3 , $J=7.3$ Hz); 2.77–2.98 (*m*, 2H, H_8), 2.77–2.98 (*m*, 1H, H_7), 3.48–3.75 (*m*, 1H, H_6), 3.77 (*s*, 3H, OCH_3 of phenyl ring), 3.88–3.93 (*q*, 2H, ester CH_2 , $J=6.7$ Hz), 4.01–4.06 (*m*, 4H, imidazolidine $2CH_2$), 5.96 (*s*, 2H, imidazolidine 2NH), 6.55 (*s*, 1H, H_{10}), 6.96–7.82 (*m*, 8H, Ar-H's); In the D_2O exchanged 1H NMR spectrum, singlet at 5.96 ppm which resonates due to 2NH's of imidazolidine moiety disappeared; ^{13}C NMR (δ ppm): 13.7 ester CH_3 , 36.0 C-7, 43.7 C-8, 49.2 C-2, and C-3, 55.2 OCH_3 of phenyl ring, 59.8 ester CH_2 , 60.9 C-6, 119.9 C-10, 120.9–158.5 Ar-C's, 159.6, 161.2 *ipso*-C, 169.2 C=O.

9-(4-chlorophenyl)-7-(4-methoxyphenyl)-1,4-diazaspiro[4.5]-deca-9-ene-6-ethyl carboxylate **30**

IR (KBr) (cm^{-1}): 3453, 3393, 3262, 3065, 2951, 2927, 2830, 1738, 1608, 1456, 1032, 824, 749, 711; 1H NMR (δ ppm): 0.92–0.94 (*t*, 3H, ester CH_3 , $J=7.2$ Hz); 2.87–3.09 (*m*, 2H, H_8), 3.12–3.19 (*m*, 1H, H_7), 3.59–3.70 (*m*, 1H, H_6), 3.71 (*s*, 3H, OCH_3 of phenyl ring), 3.87–3.92 (*q*, 2H, ester CH_2 , $J=6.7$ Hz), 4.05–4.20 (*m*, 4H, imidazolidine $2CH_2$), 5.96 (*s*, 2H, imidazolidine 2NH), 6.55 (*s*, 1H, H_{10}), 7.24–7.91 (*m*, 8H, Ar-H's); In the D_2O exchanged 1H NMR spectrum, singlet at 5.96 ppm which resonates due to 2NH's of imidazolidine moiety disappeared; ^{13}C NMR (δ ppm): 14.3 ester CH_3 , 35.6 C-7, 43.1 C-8, 48.4 C-2, and C-3, 55.2 OCH_3 of phenyl ring, 59.9 ester CH_2 , 61.0 C-6, 119.9 C-10, 120.9–158.4 Ar-C's, 159.7, 161.2 *ipso*-C, 169.1 C=O.

Microbiology

Materials

All the clinically isolated bacterial strains namely *Staphylococcus aureus*, β -Haemolytic streptococcus, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Klebsiella pneumonia* and fungal strains namely *Candida albicans*, *Candida 6*, *Candida 51*, *Candida neoformans* and *Microsporium gypseum* were obtained from Faculty of Medicine, Annamalai University, Annamalaiagar-608 002, Tamil Nadu, India.

In vitro antibacterial and antifungal activity

Minimum inhibitory concentration (MIC) in $\mu g/mL$ values was carried out by twofold serial dilution method²⁸. The respective test compounds **21–30** were dissolved in dimethylsulphoxide (DMSO) to obtain 1 mg/mL stock solution. Seeded broth (broth containing microbial spores) was prepared in NB from 24 h old bacterial cultures on nutrient agar (Hi-media, Mumbai) at $37 \pm 1^\circ C$ whereas

fungus spores from 1 to 7 days old Sabourauds agar (Hi-media, Mumbai) slant cultures were suspended in Sabourauds dextrose broth (SDB). The colony forming units (cfu) of the seeded broth were determined by plating technique and adjusted in the range of 10^4 – 10^5 cfu/mL. The final inoculum size was 10^5 cfu/mL for antibacterial assay and 1.1 – 1.5×10^2 cfu/mL for antifungal assay. Testing was performed at pH 7.4 ± 0.2 for bacteria (NB) and at a pH 5.6 for fungi (SDB). Exactly 0.4 mL of the solution of test compound was added to 1.6 mL of seeded broth to form the first dilution; 1 mL of this was diluted with a further 1 mL of seeded broth to give the second dilution and so on till six such dilutions were obtained. A set of assay tubes containing only seeded broth was kept as control. The tubes were incubated in BOD incubators at $37 \pm 1^\circ\text{C}$ for bacteria and $28 \pm 1^\circ\text{C}$ for fungi. MICs were recorded by visual observations after 24 h (for bacteria) and 72–96 h (for fungi) of incubation. Penicillin was used as standard for bacterial studies and Amphotericin B was used as standard for fungal studies.

Results and discussion

Several methods have been reported for the synthesis of imidazolidines^{29–35} which include conversion of esters using aluminium reagents²⁹, the reaction between N-ethoxy carbonylthioamides with 1,2-diamines³⁰ and the reaction of aldehydes with 1,2-diamines followed by N-halosuccinimides³¹. Recently, several methods have been developed where azalactones³², 2-aryl-1, 1-dibromoethanes³³, nitriles³⁴ and amino amides³⁵ are used as starting material for this synthesis. However, many of the synthesis protocols reported so far suffer from disadvantages such as needing anhydrous conditions²⁹, use of organic solvents^{29–35}, harsh reaction conditions²⁹, prolonged reaction time³¹, use of metals and expensive reagents²⁹ etc., Therefore the development of an alternative cost effective, safe and environment friendly reagent system is attractive. In continuation of our earlier interest in synthesizing variety of organic molecules of industrial and pharmacological interest, we attempted and succeeded to synthesize a series of imidazolidine derivatives catalyzed by activated fly ash under microwave irradiation (MWI).

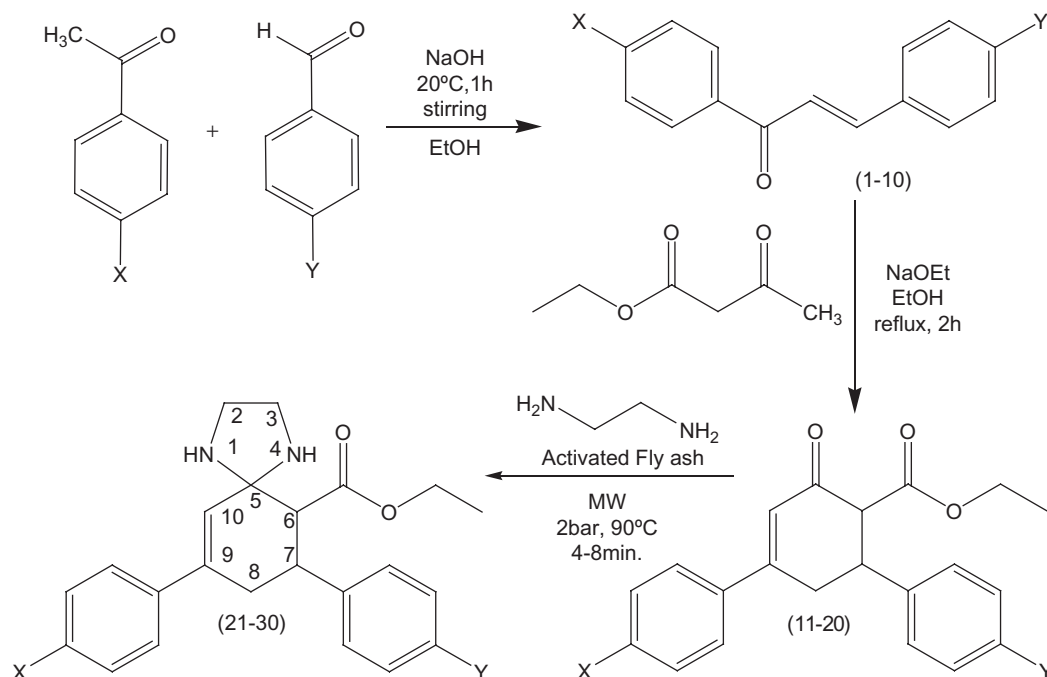
The Fly ash as-received was sieved in a 100 mesh sieve to remove any coarser and foreign particles and then mechanically ground in a ball mill to fine powder. The particle size distribution was found to be between 40 and 90 μm . Finely ground Fly ash was kept at a temperature of about 900–1000°C in a silica crucible for 1 h for activation and was used for investigation. The carbon, sulphur, and other impurities were removed by thermal activation and the resultant Fly ash is called Activated Fly ash⁴. The chemical compositions of fly ash⁴ was SiO_2 (64.03%), Al_2O_3 (15.50%), Fe_2O_3 (6.50%), CaO (4.62%), MgO (3.00%), loss on ignition (4.35%), insoluble residue (2.00%). Activated Fly ash was shown to be one of the most efficient MW absorbers with a very high specificity to MW heating. It

was able to reach a temperature of 135°C after 6 min of irradiation in domestic oven. ($p = 320 \text{ W}$). The reagent can be stored in a vacuum desiccator.

Facile synthesis of novel imidazolidine derivatives was carried out by the reaction of 6-carbomethoxy-3,5-diarylcyclohex-2-enones **11–20** with ethylene diamine in the presence of activated fly ash in dry media under scientific focused MWI, because the applications of microwave technology to rapid synthesis of biologically significant heterocyclic molecules under solvent-free conditions are very promising and numerous and has recently been recognized as a useful tool for a drug-discovery program especially in combinatorial chemistry. The reactions were performed at 90°C and two bar pressure for 4–8 min. After completion of the reaction as indicated by the TLC, the reaction mixture was poured into ice water. The mixture was extracted with dichloromethane and washed with 10% sodium bicarbonate solution, finally washed with distilled water, concentrated in rotary evaporator and purified by flash column chromatography using toluene:ethyl acetate (1:1) as eluent.

The synthetic route for the formation of target molecules **21–30** was given in Scheme 1. The physical and analytical data was given in Table 1. The structures of all the synthesized compounds **21–30** are discussed with the help of m.p.'s, elemental analysis, FT-IR, MS, one-dimensional ^1H NMR, D_2O exchanged ^1H NMR, ^{13}C NMR and two dimensional HSQC spectra. In order to investigate the spectral assignments, new imidazolidine derivative, 7-(4-fluorophenyl)-9-phenyl-1,4-diazaspiro[4.5]-deca-9-ene-6-ethyl carboxylate **23** was chosen as a representative compound. FT-IR spectrum of 7-(4-fluorophenyl)-9-phenyl-1,4-diazaspiro[4.5]-deca-9-ene-6-ethyl carboxylate **23** shows characteristic absorption frequencies at 3519, 3388, and 3262 cm^{-1} suggests the presence of NH stretching frequency. The absorption frequency at 1739 cm^{-1} is due to the presence of carbonyl stretching of ester group. Moreover, the absorption frequency at 1604 cm^{-1} is due to the presence of C=C stretching. The presence of band at 1443 cm^{-1} (C-N) is more evident for the formation of **23**. The observed NH stretching, C=C stretching, C=O of ester functional group absorption bands, all support evidence for the formation of compound **23**. The mass spectrum of **23** shows molecular ion peak at m/z 381 ($\text{M}^{++}+1$) which is consistent with the proposed molecular formula, $\text{C}_{23}\text{H}_{25}\text{N}_2\text{O}_2\text{F}$. Elemental analysis [C_{cal} 72.61, C_{obs} 72.52; H_{cal} 76.62, H_{obs} 6.57; N_{cal} 7.36, N_{obs} 7.23] is consistent with the proposed molecular formula [$\text{C}_{23}\text{H}_{25}\text{N}_2\text{O}_2\text{F}$] of **23**.

The assignments of signals in ^1H NMR spectrum of **23** have been done based on total widths, position, and spin multiplicities. A singlet observed at 6.57 ppm is conveniently assigned to H10 proton of cyclohexene moiety. Two multiplets appeared in the region of 2.74–2.94 ppm is due to methylene protons H8 of cyclohexene moiety. Another multiplet observed in the range 2.98–3.13 ppm is due to benzylic proton H7. The methyl protons (CH_3) of ester group appear as a triplet, centered at 0.89 ppm.



Scheme 1. Fly ash catalyzed facile synthesis of novel spiro imidazolidine derivatives under microwave irradiation.

Table 1. Physical and analytical data for the title compounds **21–30**.

Compounds	X	Y	m.p.(° C)	MW	Time (min)	Yield (%)	Elemental analysis (%)			m/z (M+1)+•Molecular formula
							C Found (calculated)	H Found (calculated)	N Found (calculated)	
21	H	H	77	6	95		76.11 (76.21)	7.15 (7.23)	7.63 (7.73)	363 C23H26N2O2
22	H	Cl	100	8	93		69.48 (69.60)	6.24 (6.35)	6.95 (7.06)	397 C23H25N2O2Cl
23	H	F	65	4	98		72.52 (72.61)	6.57 (6.62)	7.23 (7.36)	381 C23H25N2O2F
24	H	CH3	117	5	90		76.49 (76.56)	7.43 (7.50)	7.32 (7.44)	377 C24H28N2O2
25	H	OCH3	112	8	88		73.33 (73.44)	7.10 (7.19)	7.01 (7.14)	393 C24H28N2O3
26	Cl	H	68	7	91		69.52 (69.60)	6.30 (6.35)	6.97 (7.06)	397 C23H25N2O2Cl
27	OCH3	H	65	6	90		73.32 (73.44)	7.11 (7.19)	7.03 (7.14)	393 C24H28N2O3
28	Cl	CH3	73	4	93		70.04 (70.15)	6.53 (6.62)	6.78 (6.82)	411 C24H27N2O2Cl
29	OCH3	Cl	69	6	90		67.42 (67.52)	6.25 (6.37)	6.51 (6.56)	427 C24H27N2O3Cl
30	Cl	OCH3	99	8	90		67.44 (67.52)	6.29 (6.37)	6.48 (6.56)	427 C24H27N2O3Cl

The methylene protons (CH_2) of ester group observed as a quartet, centered at 3.90 ppm. A signal observed as multiplet at around 3.56–3.88 ppm corresponding to one proton is unambiguously assigned to H6 proton. The two NH protons of imidazolidine moiety were appeared as broad singlet at 6.02 ppm corresponding to two protons. In addition, methylene protons (H-2, H-3) at C-2 and C-3 of imidazole moiety observed as a multiplet at around 3.95–4.13 ppm. The aromatic protons appear as a multiplet in the range 6.83–7.94 ppm. The presence of labile-NH protons at positions one and four is confirmed by recording the ^1H NMR spectrum after adding D_2O . The peak at 6.02 ppm due to 2 NH's protons at position 1 and 4 have disappeared in the D_2O exchanged ^1H NMR spectrum. In ^{13}C NMR spectrum of **23** six resonances in the aliphatic region 13.7, 59.8, 48.4, 59.8, 35.2, and 42.9 ppm have been observed. (with the help of HSQC the individual assignment could be carried out). The remaining ^{13}C resonances at 169.1, 157.8, and 158.2 ppm are due to

quaternary carbons. The ^{13}C resonance at 122.3 ppm may be due to C-10 carbon. Aromatic carbons are observed in the range of 113.1–136.1 ppm.

In the HSQC spectrum of **23**, the one bond correlation (13.7/0.90 ppm) between methyl protons and methyl carbon of ester confirms that signal observed at 0.90 ppm must be due to methyl protons of ester and ^{13}C resonance at 13.7 ppm must be assigned to methyl carbon of ester. A multiplet observed in the region of 2.74–2.94 ppm is assigned to methylene protons H8. Because H8 proton is assigned from HSQC spectrum, the cross peak (42.9/2.74–2.94 ppm) confirms that the ^{13}C resonance at 42.9 ppm is due to C-8 carbon. In HSQC, the ^{13}C resonances at 35.2 ppm has correlations with benzylic proton H7 (35.2/2.98–3.13 ppm) hence C-7 resonates at 35.2 ppm and multiplet observed at around 2.98–3.13 ppm must be due to benzylic proton H7. In HSQC the ^{13}C resonances at 59.8 ppm has correlations with the methylene protons of ester group (59.8/3.89 ppm) and hence methylene

carbon of ester resonates at 59.8 ppm. The ^{13}C resonance at 122.3 ppm shows cross peak (122.3/6.57 ppm) with H10 proton and hence that resonance has been assigned to C-10. The ^{13}C resonance at 61.0 ppm has cross peak (61.0/3.56–3.88 ppm) in HSQC with methine proton signal (H6). Hence the resonance at 61.0 ppm must be due to C-6 carbon. Moreover, the ^{13}C resonance at 48.4 ppm has correlation with a multiplet observed in range 3.95–4.13 ppm (48.4/3.95–4.13 ppm). The multiplet observed at around 3.95–4.13 ppm is unambiguously assigned to methylene protons at C-2 and C-3. The cross peaks (48.4/3.95–4.13 ppm) confirm that ^{13}C resonances at 48.4 ppm must be due to methylene carbons at C-2 and C-3 of imidazole moiety. In HSQC, the ^{13}C resonances at 80.2, 157.8, 158.2, and 169.1 ppm have no correlations with protons. Among the carbon resonances, the ^{13}C resonances at 169.1 ppm must be due to carbonyl carbon of ester and ^{13}C resonance at 80.2 ppm is due to spiro carbon (C-5). The ^{13}C resonances at 157.8 and 158.2 ppm are assigned to *ipso* carbons. The C-9 carbon resonance is merged with the aromatic region (157.8 and 158.2 ppm).

In vitro antibacterial activity

Novel spiro imidazolidine derivatives **21–30** are tested for their *in vitro* antibacterial activity against *S.aureus*, β -*H.streptococcus*, *M.luteus*, *P.aeruginosa*, *P.vulgaris*, and *K.pneumonia*. Penicillin is used as standard drug. MIC in $\mu\text{g/mL}$ values is reproduced in Table 2. Among the compounds tested, compound **21** which is having no substitution at the *para* position of phenyl rings attached to C-7 and C-9 carbons of cyclohexene moiety exerted moderate activities against all the used bacterial strains. All the synthesized compounds **21–30** exhibit a wide range of antibacterial potency against the tested strains except compounds **21** and **26** which did not show activity against β -*H.streptococcus* and *M.luteus* even at a higher concentration of 200 $\mu\text{g/mL}$. Structure-activity relationship results for the synthesized compounds have shown that compounds **22** and **23** which are having electron withdrawing chloro/fluoro substitution at the *para* position of phenyl ring attached to C-7 of cyclohexene moiety, respectively show excellent antibacterial activity against *P.aeruginosa* at a MIC value of 6.25 $\mu\text{g/mL}$. Compound **24**, which is having electron donating methyl substitution at the *para* position of phenyl ring attached to C-7 of cyclohexene moiety respectively show excellent antibacterial activity against *S.aureus* and *K.pneumonia* at a MIC value of 6.25 $\mu\text{g/mL}$. Compound **25**, which has electron donating methoxy substitution at the *para* position of phenyl ring attached to C-7 of cyclohexene moiety exerted good activities against *S.aureus*, *M.luteus*, and *P.vulgaris* at a MIC value of 12.5 $\mu\text{g/mL}$, whereas it exhibit excellent activity against *K.pneumonia* at a MIC value of 6.25 $\mu\text{g/mL}$. Besides these, compound **26**, which has electron withdrawing chloro substitution at the *para* position of phenyl ring attached to C-9 of cyclohexene moiety show admirable antibacterial activity against *P.aeruginosa* at a MIC value of 12.5 $\mu\text{g/mL}$. Replacement

of chloro substitution by methoxy substitution at the *para* position of phenyl ring attached to C-9 of cyclohexene moiety exhibit tremendous antibacterial activity against *S.aureus* and β -*H.streptococcus* at a MIC value of 6.25 $\mu\text{g/mL}$. Compound **28**, which have both electron withdrawing chloro substitution at the *para* position of phenyl ring attached to C-9 and electron donating methyl substitution at the *para* position of phenyl ring attached to C-7 position of cyclohexene moiety exhibit good activity against *P.aeruginosa* and *K.pneumonia* at a MIC value of 12.5 $\mu\text{g/mL}$. Likewise, compound **29** which have electron donating methoxy substitution at the *para* position of phenyl ring attached to C-9 position and electron withdrawing chloro substitution at the *para* position of phenyl ring attached to C-7 of cyclohexene moiety possess tremendous activity against *M.luteus*, *P.vulgaris*, and *K.pneumonia* at a MIC value of 6.25 $\mu\text{g/mL}$. Compound **30**, which have both electron withdrawing chloro substitution at the *para* position of phenyl ring attached to C-9 and electron donating methoxy substitution at the *para* position of phenyl ring attached to C-7 position of cyclohexene moiety exhibit good activity against β -*H.streptococcus* and *M.luteus* and exerted excellent activity against *P.vulgaris* at a MIC value of 12.5 $\mu\text{g/mL}$.

In vitro antifungal activity

In vitro antifungal activity of compounds **21–30** is studied against the fungal strains viz., *Candida albicans*, *Candida 6*, *Candida 51*, *Candida neoformans*, and *Microsporum gypseum*. Amphotericin B is used as a standard drug. MIC in $\mu\text{g/mL}$ values is reproduced in Table 3. Compounds **21–30**, irrespective of the nature of functional group electron donating (CH_3/OCH_3) or (electron withdrawing (F/Cl) at the phenyl rings exerted moderate *in vitro* antifungal activity against all the tested fungal strains in the range of 6.25–200 $\mu\text{g/mL}$ except compounds **21**, and **27** which do not show any activity against the tested fungal strains namely *M.gypseum* and compounds **22** and **26** against *C.albicans* even at a higher concentration of 200 $\mu\text{g/mL}$. Compounds **22** and **24** possess good antifungal activity against *M.gypseum* and *Candida 51*, respectively at a MIC value of 12.5 $\mu\text{g/mL}$. Fluoro substituted compound **23** shows excellent antifungal activity against *M.gypseum* at a MIC value of 6.25 $\mu\text{g/mL}$. Methoxy substituted compound **25** exhibits good activity against *C.albicans* and *C.neoformans* at a MIC value of 12.5 $\mu\text{g/mL}$, whereas it show tremendous activity against *Candida 6* at a MIC value of 6.25 $\mu\text{g/mL}$. Compound **26** show moderate activity against all the tested fungal strains except against *M.gypseum*, which shows activity at a MIC value of 12.5 $\mu\text{g/mL}$. Compound **27** possess good activity against *C.albicans* and *Candida 6* at a MIC value of 12.5 $\mu\text{g/mL}$, whereas compound **28** exerted good activity against *C.albicans* and *Candida 51* at a MIC value of 12.5 $\mu\text{g/mL}$ and it shows excellent activity against *C.neoformans* at a MIC value of 6.25 $\mu\text{g/mL}$. Good antifungal activity is exhibited by compound **29**

Table 2. *In vitro* antibacterial activity of compounds **21-30** against clinically isolated bacterial strains.

Compounds	X	Y	Minimum inhibitory concentration (MIC) in µg/mL					
			<i>S.aureus</i>	β -Haemolytic streptococcus	<i>M.luteus</i>	<i>P.aeruginosa</i>	<i>P.vulgaris</i>	<i>K.pneumonia</i>
21	H	H	100	‘-’	200	50	100	200
22	H	Cl	200	100	100	6.25	50	25
23	H	F	100	50	50	6.25	50	25
24	H	CH ₃	6.25	12.5	25	200	25	6.25
25	H	OCH ₃	12.5	50	12.5	200	12.5	6.25
26	Cl	H	100	50	‘-’	12.5	200	50
27	OCH ₃	H	6.25	6.25	50	100	50	200
28	Cl	CH ₃	100	25	50	12.5	25	12.5
29	OCH ₃	Cl	12.5	25	6.25	25	6.25	6.25
30	Cl	OCH ₃	25	12.5	12.5	50	6.25	25
Penicillin			25	25	50	25	50	25

‘-’ No inhibition even at a higher concentration of 200 µg/mL.

Table 3. *In vitro* antifungal activity of compounds **21-30** against clinically isolated fungal strains.

Compound	X	Y	Minimum inhibitory concentration (MIC) in µg/mL				
			<i>C.albicans</i>	Candida 6	Candida 51	Candida neoformans	<i>M.gypseum</i>
21	H	H	100	200	100	100	‘-’
22	H	Cl	‘-’	200	50	200	12.5
23	H	F	200	100	200	50	6.25
24	H	CH ₃	25	25	12.5	25	50
25	H	OCH ₃	12.5	6.25	25	12.5	25
26	Cl	H	‘-’	200	25	200	12.5
27	OCH ₃	H	12.5	12.5	25	25	‘-’
28	Cl	CH ₃	12.5	25	12.5	6.25	50
29	OCH ₃	Cl	6.25	6.25	25	12.5	12.5
30	Cl	OCH ₃	6.25	12.5	50	100	12.5
Amphotericin B			25	50	25	50	25

‘-’ No inhibition even at a higher concentration of 200 µg/mL.

against *C.neoformans* and *M.gypseum* and compound **30** exhibit activity against *Candida* 6 and *M.gypseum* at a MIC value of 12.5 µg/ mL. In addition, excellent antifungal activity is noted against *C.albicans* and *Candida* 6 by compound **29** and against *C.albicans* by compound **30** at a MIC value of 6.25 µg/ mL.

Conclusion

An array of novel spiro imidazolidine derivatives are synthesized by in the presence of activated fly ash in *dry media* under scientific MWI and the structures of the products are characterized by melting point, elemental analysis, MS, FT-IR, one-dimensional NMR (¹H, D₂O exchanged ¹H & ¹³C) and two dimensional Heteronuclear Single Quantum Coherence (HSQC) spectroscopic data. In addition, the title compounds are screened for their anti-microbial activities against a spectrum of clinically isolated antibacterial and antifungal strains. The microbiological screening studies carried out to evaluate the antibacterial and antifungal potencies of the novel spiro imidazolidine derivatives **21-30** are clearly known from Table 2 and Table 3. Structure-activity relationship results revealed that compounds **22, 23** against *P.aeruginosa*, **24** against *S.aureus*, **24, 25** against *K.pneumonia*, **27** against *S.aureus*, β -H.streptococcus, **29** against *M.luteus*,

K.pneumonia, **29, 30** against *P.vulgaris* exhibited excellent antibacterial activity at a MIC value of 6.25 µg/mL. Results of the antifungal activity study show that the nature of substituents on the phenyl ring viz., fluoro, chloro, methyl, methoxy, functions at the *para* positions of the aryl ring of cyclohexane moieties are determinant for the nature and extent of the antifungal activity of all the synthesized compounds **19-27** over all fungal strains. Compound **23** against *M.gypseum*, **25** against *Candida* 6, **29** against *Candida* 6 and **29, 30** against *C.albicans* revealed excellent antifungal activity at a MIC value of 6.25 µg/mL. These observations may promote a further development of our research in this field. Further development of this group of novel spiro imidazolidine derivatives may lead to compounds with better pharmacological profile than standard antibacterial and antifungal drugs.

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