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ORIGINAL ARTICLE

In vitro biological studies and structural elucidation of fluoro-substituted phenyl acrylic acids

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Abstract

Background: Synthesis, characterization, and biological significance of different substituted phenyl acrylic acids have been studied. These acids are contributing a key role during synthesis of antimicrobial drugs. *Method*: The acids were prepared by condensation of various substituted phenyl acetic acids and aldehydes. o-Piperonal was prepared using 2,3-methylenedioxy benzaldehyde. These phenyl acrylic acids were characterized by using analytical techniques, for example, Fourier transform infrared spectroscopy, multinuclear NMR (^{1}H, ^{13}C NMR), and GC-MS. The structure of crystalline products was also elucidated by X-ray crystallography. Small intermolecular and intramolecular weaker interactions were also studied by these characterization techniques. *Results*: The geometry of compounds in solid and solution state has been studied. The presence of weaker interactions in these molecules is also observed which may increase the hydrolysis and lipophilicity and ultimately improve the activity against different microbes. *Conclusion*: The effect of different groups attached to the main rings and the presence of smaller interaction have been studied. Their factors may enhance the activity of these compounds.

Key words: *Biological activity; characterization; hydrogen bonding; phenyl acrylic acids; X-ray*

Introduction

The appearance of bacterial resistance continues to be a growing problem in the treatment of bacterial infections. The particular problem is in multidrug resistance for a variety of pathogens such as *Staphylococcus aureus* and *Streptococcus pneumonia*, and their control is a matter of great concern. Phenyl acrylic acids are contributing a significant role in the pharmaceutical industry because of their extensive applications. These compounds are used as starting materials for the synthesis of antimicrobial drugs. The presence of a fluoro group has been shown to increase their effectiveness in prototype medicinals[1]. Derivatives of these acids have been observed in shikimic acid metabolic pathways in higher plants[2]. A great deal of research has been devoted to the study of complexes of these acids, especially those including sulfur and nitrogen compounds. These are commonly used as anti-inflammatory, antibiotic, antiallergic, and antioxidant agents[3]. The most significant application of these acids includes the synthesis of HCV NS5B polymerase inhibitors[4].

These compounds can be prepared by special organic methods, for example, Reformatszkij, Suzuki, Wadswort–Emmons, and Witting reactions. Another most significant reaction type is the Perkin reaction[5]. Here, aromatic aldehydes react with acetic acid derivatives under basic conditions, providing substituted cinnamic acids with high *E*-selectivity. Acids synthesized by this method frequently demonstrate intermolecular hydrogen bonding that leads to dimeric structures. These smaller interactions also enhance the hydrolysis of these compounds that favors the permeation through lipid layer and hence improve the biological activity. In the analysis of these compounds and their in vitro antimicrobial studies, we

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have synthesized and characterized a number of different fluoro-substituted phenyl acrylic acids. Selected compounds have been tested against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Bordetella bronchiseptica* bacterial strains and *Saccharomyces cerevisiae* fungal strain. These compounds have shown a comparable activity against reference drugs. The characterization of these acids was accomplished with analytical techniques, for example, IR spectroscopy, mass spectrometry, and single crystal X-ray analysis in the solid state and multinuclear nuclear magnetic resonance (NMR) (^{1}H, ^{13}C) spectroscopy in solution.

Experimental

Materials

All aldehydes, phenyl acetic acids, and potassium carbonate were purchased from Aldrich and used without further purification. The solvent utilized was acetic anhydride that was prepared by using a standard procedure[6].

Instrumentation

Melting points were determined in a capillary tube using electro-thermal melting point apparatus model MPD Mitamura Riken Kogyo (Japan). Fourier-transformed infrared spectra (FTIR) were recorded as KBr discs on a Bio-Rad Excaliber FTS Model 3000 MX in the frequency range of 4000–400 cm^{-1}. ^{1}H and ^{13}C NMR were recorded on a Bruker 300 MHz FT-NMR spectrometer using $CDCl_3$ as an internal reference. The title compounds were analyzed by Gas chromatograph–Mass Spectroscopy (GC–MS) 6890N Agilent technologies (Santa Clara, CA, USA). Single-crystal X-ray diffraction analysis was carried out on a Bruker Apex 2 diffractometer, which is a three-circle diffractometer.

*Synthesis of 2,3-methylendioxy benzaldehyde (*o*-piperonal)*

A mixture of 3.0 g (22 mmol) 2,3-dihydroxy benzaldehyde, 4.6 g (27.5 mmol) dibromomethane, 3.1 g (23 mmol) potassium carbonate, and 2.0 g cupric oxide in 200 mL of DMF was heated under inert atmosphere at 130°C for 2.5 hours. The cooled mixture was diluted with 65.0 mL of water and extracted with four equal portions of 50 mL benzene. The benzene extract was washed with water to remove the impurities and dried over anhydrous potassium carbonate. Distillation under reduced pressure provided 3.5 g of black residue. Further vacuum distillation of the black residue was carried out to get 2.6 g of light yellow oil that turned crystalline as it solidified: melting point 35–36°C and yield 60% (Figure 1).

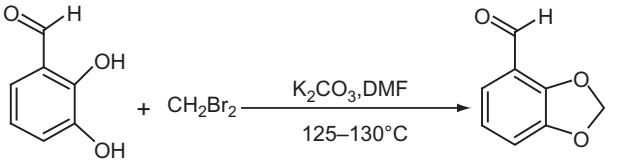
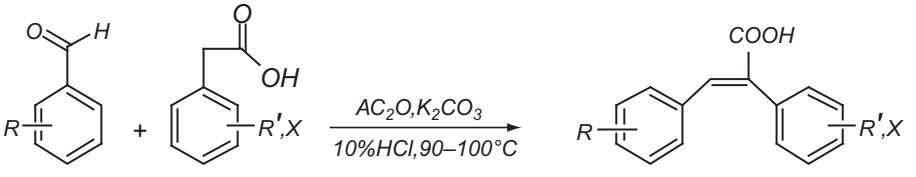


Figure 1. The preparation of *o*-piperonal.

Synthesis of phenyl acrylic acids

The acids were prepared by the literature method[1]. The methodology of synthesis is described as follows[7,8].

The reaction was carried out by dissolving equimolar amounts of respective aldehydes and substituted phenyl acetic acids in 10 mL acetic anhydride that acts as a dehydrating agent. The basic medium was provided by including K_2CO_3 dissolved in water. Following the additions, the temperature was slowly raised to 90–100°C and these conditions were sustained for 24 hours. After that, 10% HCl and 20 mL of H_2O were added to hot solution and an additional 2 hours of stirring was carried out at cold conditions. The precipitated product was filtered off and washed with water repeatedly to ensure the complete removal of traces of acetic acid and acetic anhydride. Crystals suitable for X-ray analysis were grown by dissolving 0.5 g of the compound in a minimum amount of chloroform (5.0 mL) to which another suitable solvent (e.g., *n*-hexane) was also added. Slow evaporation of the solvent at room temperature over days yielded fine crystals that were subsequently washed with acetone (Figure 2).



R = *o*-O_2CH_2, *p*-CF_3, *o*-CH_3; R = *p*-OCH_3, *m*-CH_3; X = *p*-F, *o*-F, *p*-Cl, *p*-Br

Figure 2. The preparation of substituted phenyl acrylic acids.

Spectral data of compounds

(*E*)-3-(2-Methylphenyl)-2-(4-fluorophenyl) acrylic acid (1)

Synthesis and spectral data. 4-Fluorophenyl acetic acid 0.5 g (3.25 mmol), *o*-tolualdehyde 0.39 g (3.25 mmol), potassium carbonate 0.25 g (1.8 mmol). Crystalline product obtained.

Analytical data. $C_{16}H_{13}O_2F$, molecular mass 256, melting point 167–169°C, yield 80%. IR data: (KBr, cm^{-1}) υ_{C-H} 3000 s, υ_{O-H} 2514–3115 b, $\upsilon_{asym\ C=O}$ 1670 s, $\upsilon_{C=C}$ 1487 s, $\upsilon_{sym\ C=O}$ 1423, $\Delta\upsilon = \upsilon_{asym} - \upsilon_{sym}$ 247, υ_{C-F} 700–800 b. ^{1}H NMR data: ($CDCl_3$, 293K) δ ppm 10.48 s, 1H (*acidic proton*), 8.19 s, 1H (*acrylic proton*), 7.15–7.21 m, 3H (*aromatic protons*), 6.94 dd, 2H (*aromatic protons*) ^1J[^{19}F, ^{1}H] 7.8 Hz ^1J[^{1}H, ^{1}H] 6.0 Hz, 6.91 t(8.4), 2H (*aromatic proton*), 6.79 d(7.8), 1H (*aromatic proton*), 2.41 s, 3H (*aromatic substituted methyl proton*). ^{13}C NMR data: ($CDCl_3$, 293K) δ ppm 173.1 (*acidic carbon*), 163.7 (*aromatic carbon*) ^1J[^{13}C, ^{19}F] 246 Hz, 132.1 (*aromatic carbons*) ^3J[^{13}C, ^{19}F] 8.3 Hz, 115.8 (*aromatic carbons*) ^2J[^{13}C, ^{19}F] 21.8 Hz, (141.8, 137.9, 133.6, 131.5, 130.7, 129.7, 125.5, *acrylic and aromatic carbons*). Mass fragmentation, *m/z* (%): [$C_{16}H_{13}O_2F$] 256 (100), [$C_{15}H_{10}O_2F$] 241(29), [$C_{15}H_{12}F$] 211(36), 1[$C_{14}H_9F$] 96(49), [C_9H_7] 115(32), [C_7H_7] 91(16).

(*E*)-3-(4-Triflouromethylphenyl)-2-(4-flourophenyl) acrylic acid (2)

Synthesis and spectral data. 4-Fluorophenyl acetic acid 0.5 g (3.25 mmol), 4-trifluoromethyltolualdehyde 0.56 g (3.25 mmol), potassium carbonate 0.25 g (1.8 mmol). Crystalline product obtained.

Analytical data. $C_{16}H_{10}O_2F_4$, molecular mass 310, melting point 163–166°C, yield 78%. IR data: (KBr, cm^{-1}) υ_{C-H}, 3150 s, υ_{O-H} 3150–2506 b, $\upsilon_{asym\ C=O}$ 1679 s, $\upsilon_{C=C}$ 1511 sharp, $\upsilon_{sym\ C=O}$ 1416, $\Delta\upsilon = \upsilon_{asym} - \upsilon_{sym}$ 263, υ_{C-F} 800–700 b. ^{1}H NMR data: ($CDCl_3$, 293K) δ ppm 7.98 s, 1H (*acrylic proton*), 7.49 d(8.4), 2H (*aromatic protons*), 7.28–7.18 m, 4H (*aromatic protons*), 7.14 dd, 2H (*aromatic protons*) ^1J[^{19}F, ^{1}H] 8.7 Hz ^1J[^{1}H, ^{1}H] 6.6 Hz. ^{13}C NMR data: ($CDCl_3$, 293K) δ ppm 172.6 (*acidic carbon*), 164.4 (*C–F aromatic carbon*) ^1J[^{13}C-^{19}F] 246.7 Hz, 116.2 (*aromatic cartbon*) ^2J[^{13}C, ^{19}F] 21.8 Hz, 131.7 (*aromatic carbon*) ^2J[^{13}C, ^{19}F) doublet 8.3 Hz, 130.4 (*aromatic carbon*) ^3J[^{13}C, ^{19}F] doublet 3.8 Hz, 125.5 ^1J[^{13}C, ^{19}F] quartet 26.8 Hz (141.0, 137.6, 132.9, 131.2, 130.8, 129.1, *acrylic and aromatic carbons*).

(*E*)-3-(4-Trifluoromethylphenyl)-2-(3-methylphenyl) acrylic acid (3)

Synthesis and spectral data. 3-Methylphenyl acetic acid 0.5 g (3.33 mmol), 4-trifluoromethyltolualdehyde 0.58 g (3.33 mmol), potassium carbonate 0.25 g (1.8 mmol). Crystalline product obtained.

Analytical Data. $C_{17}H_{13}O_2F_3$, molecular mass 306, melting point 185–188°C, yield 76%. IR data: (KBr, cm^{-1}) υ_{C-H} 3150 s, υ_{O-H} 2628–3120 b, $\upsilon_{asym\ C=O}$ 1679 s, $\upsilon_{C=C}$ 1486 s, $\upsilon_{sym\ C=O}$ 1421, $\Delta\upsilon = \upsilon_{asym} - \upsilon_{sym}$ 258, υ_{C-F} 800–700 b. ^{1}H NMR data: ($CDCl_3$, 293K) δ ppm 9.30 s, 1H (*acidic proton*), 7.94 s, 1H (*acrylic proton*), 7.46 d(8.4), 2H (*aromatic proton*), 7.32 d(7.5), 2H (*aromatic proton*), 7.28 d(1.5), 1H (*aromatic proton*), 7.20 t(7.8), 1H (*aromatic proton*), 7.06 bs, 2H (*aromatic protons*), 2.36 s, 3H (*aromatic-substituted methyl protons*). ^{13}C NMR data: ($CDCl_3$, 293 K) δ ppm 172.8 (*acidic carbon*), 130.8 (*aromatic carbon*) ^2J[^{13}C, ^{19}F) doublet 12.8 Hz, 125.6 (*aromatic carbon*) ^1J[C–F] quartet 37.5 Hz (140.3, 138.6, 137.8, 134.5, 130.0, 121.9, 130.6, 128.8, 129.3, *acrylic and aromatic carbons*), 21.4 (*aromatic-substituted methyl carbon*). Mass fragmentation, *m/z* (%): [$C_{17}H_{13}O_2F_3$] 306 (100), [$C_{17}H_{13}O_2F_2$] 287 (19), [$C_{16}H_{11}O_2F_3$] 273 (22), [$C_{16}H_{12}F_3$] 261 (46), [$C_{15}H_9F_3$] 246 (28), [$C_{10}H_6O_2F_2$] 196 (6), [C_7H_7] 91 (5), [C_6H_4] 76 (4).

(*E*)-3-(4-Triflouromethylphenyl)-2-(4-bromophenyl) acrylic acid (4)

Synthesis and spectral data. 4-Bromophenyl acetic acid 0.5 g (2.3 mmol), 4-triflouromethyltolualdehyde 0.40 g (2.3 mmol), potassium carbonate 0.18 g (1.3 mmol). Crystalline product obtained.

Analytical data. $C_{16}H_{10}O_2F_3Br$, molecular mass 371, melting point 177–179°C, yield 76 %. IR data: (KBr, cm^{-1}) υ_{C-H} 3000 s, υ_{O-H} 3100–2500 b, $\upsilon_{asym\ C=O}$ 1692 s, $\upsilon_{C=C}$ 1487 s, $\upsilon_{sym\ C=O}$ 1419, $\Delta\upsilon = \upsilon_{asym} - \upsilon_{sym}$ 247, υ_{C-F} 800–700 b. ^{1}H NMR data: ($CDCl_3$, 293K) δ ppm 7.93 s, 1H (*acrylic proton*), 7.48 d(8.4), 2H (*aromatic protons*), 7.14–7.38 m, 6H (*aromatic protons*). ^{13}C NMR data: ($CDCl_3$, 293K) δ ppm 171.9 (*acidic carbon*), 133.1 (*aromatic carbon*) ^2J[^{13}C, ^{19}F] doublet 6.0 Hz, 125.5 (*aromatic carbon*) ^1J[^{13}C, ^{19}F] quartet 30 Hz, (140.7, 137.5, 134.5, 131.8, 131.2, 130.8, 130.7, 129.2, *acrylic and aromatic carbons*). Mass fragmentation, *m/z* (%): [$C_{16}H_9O_2F_3Cl$] 310 (100), [$C_{16}H_9F_3$] 281 (5), [$C_{16}H_{10}O_2F_3$] 291 (14.8), [$C_{15}H_8F_3$] 245 (25), [$C_{10}H_6O_2F_2$] 196 (42), [$C_{10}H_6O_2F$] 177 (7), [C_8H_5Cl] 136.5 (34), [C_7H_7] 91 (5), [C_6H_4] 76 (4).

(*E*)-3-(4-Triflouromethylphenyl) -2-(4-methoxyphenyl) acrylic acid (5)

Synthesis and spectral data. 4-Methoxyphenyl acetic acid 0.5 g (3.0 mmol), 4-triflouromethyltolualdehyde 0.52 g (3.0 mmol), potassium carbonate 0.23 g (1.7 mmol). Crystalline product obtained.

Analytical data. $C_{17}H_{13}O_3F_3$, molecular mass 322, yield 76%. IR data: (KBr, cm^{-1}) υ_{C-H} 2965 s, υ_{O-H} 3150–2533 b, $\upsilon_{asym\ C=O}$ 1685 s, $\upsilon_{C=C}$ 1511 s, $\upsilon_{sym\ C=O}$ 1416, $\Delta\upsilon = \upsilon_{asym} - \upsilon_{sym}$ 269, υ_{C-F} 800–700 b. ^{1}H NMR data: ($CDCl_3$, 293 K) δ ppm 8.30 s, 1H (*acrylic proton*), 7.68 s, 1H (*aromatic proton*), 7.33 d(8.1), 2H (*aromatic protons*), 7.02–6.75 m, 6H (*aromatic protons*), 3.73 s, 3H (*methoxy protons*). ^{13}C

NMR data: ($CDCl_3$, 293 K) δ ppm 173.1 (*acidic carbon*), 159.3 (*methoxy-substituted aromatic carbon*) (138.5, 138.1, 130.9, 129.2, 127.5, 121.9, 114.1, *acrylic and aromatic carbons*), 130.5 (*aromatic carbon*) $^2J[^{13}C, ^{19}F]$ doublet 32 Hz, 125.6 (*aromatic carbon*) $^1J[^{13}C, ^{19}F]$ quartet 48.8 Hz, 55.4 (*aromatic-substituted methoxy carbon*).

(*E*)-3-(4-Triflouromethylphenyl)-2-(4-chlorophenyl) acrylic acid (6)

Synthesis and spectral data. 4-Chlorophenyl acetic acid 0.5 g (2.94 mmol), 4-triflouromethyltolualdehyde 0.51 g (2.94 mmol), potassium carbonate 0.23 g (1.7 mmol). Crystalline product obtained.

Analytical data. $C_{16}H_{10}O_2F_3Cl$, molecular mass 326.5, melting point 162–165°C, yield 75%. IR data: (KBr, cm^{-1}) υ_{C-H} 3000 s, υ_{O-H} 3100–2500 b, $\upsilon_{asym\ C=O}$ 1692 s, $\upsilon_{C=C}$ 1487 s, $\upsilon_{sym\ C=O}$ 1419 s, $\Delta\upsilon = \upsilon_{asym} - \upsilon_{sym}$ 247, $\upsilon_{O_2-CH_2}$ 932 s. ^{1}H NMR data: ($CDCl_3$, 293K) δ ppm 7.99 s, 1H (*acrylic proton*), 7.63 d(8.1), 2H (*aromatic protons*), 7.51 d(8.4), 2H (*aromatic protons*), 7.28 d(8.1), 2H (*aromatic protons*), 7.11 d(8.4), 4H (*aromatic protons*). ^{13}C NMR data: ($CDCl_3$, 293K) δ ppm 172.3 (*acidic carbon*), 125.5 (*aromatic carbon*) $^1J[^{13}C, ^{19}F]$ quartet 26 Hz (141.2, 137.4, 122.9, 132.1, 133.2, 131.9, 131.5, 130.9, 128.63, *acrylic and aromatic carbons*). Mass fragmentation, *m/z* (%): $[C_{16}H_{10}O_2F_3Br]$ 371.0 (89), $[C_{16}H_9O_2F_3Br]$ 370 (88), $[C_{16}H_9O_2F_2Br]$ 351 (9), $[C_{16}H_9O_2F_3]$ 291 (17), $[C_{15}H_9F_3]$ 246 (100), $[C_{10}H_6O_2F_2]$ 196 (33), $[C_{10}H_7O_2F]$ 178 (7), $[C_6H_3]$ 75 (12).

(*E*)-3-(2,3-Methylenedioxyphenyl) -2-(4-flourophenyl) acrylic acid (7)

Synthesis and spectral data. 2-Fluorophenyl acetic acid 0.5 g (3.25 mmol), *o*-piperonal 0.49 g (3.25 mmol), potassium carbonate 0.25 g (1.8 mmol). Crystalline product obtained.

Analytical data. $C_{16}H_{11}O_4F$, molecular mass 286, melting point 173–176°C, yield 88%. IR data: (KBr, cm^{-1}) υ_{C-H} 3063 s, υ_{O-H} 2900–2512 b, $\upsilon_{asym\ C=O}$ 1669 s, $\upsilon_{C=C}$ 1508 s, $\upsilon_{sym\ C=O}$ 1420, $\Delta\upsilon = \upsilon_{asym} - \upsilon_{sym}$ 249, υ_{C-F} 800–700 b. ^{1}H NMR data: ($CDCl_3$, 293K) δ ppm 7.19 t(9.0), 2H (*aromatic protons*), 7.30 d(8.4), 2H (*aromatic protons*), 7.33 s, 1H (*acrylic proton*), 6.0 s, 2H (*methylenedioxy protons*), 6.60–6.78 m, 3H (*aromatic protons*). ^{13}C NMR data: ($CDCl_3$, 293K) δ ppm 167.5 (*carbonyl carbon*), 163.7 (*C–F aromatic carbon*) $^1J[^{13}C, ^{19}F]$ 246 Hz, 115.8 (*aromatic carbons*) $^2J[^{13}C, ^{19}F]$ doublet 21.8 Hz, 131.9 (*aromatic carbons*) $^3J[^{13}C, ^{19}F]$ doublet 7.5 Hz, 147.6 (*methylenedioxy carbons*) (133.0, 132.6, 132.1, 121.1, 121.3, 117.0, 108.7, 101.4, *acrylic and aromatic carbon*).

(*E*)-3-(2,3-Methylenedioxyphenyl)-2-(2-flourophenyl) acrylic acid (8)

Synthesis and spectral data. 4-Fluorophenyl acetic acid 0.5 g (3.25 mmol), *o*-piperonal 0.49 g (3.25 mmol), potassium carbonate 0.25 g (1.8 mmol). Crystalline product obtained.

Analytical data. $C_{16}H_{11}O_4F$, molecular mass 286, melting point 203–205°C, yield 83%. IR data: (KBr, cm^{-1}) υ_{C-H} 3110 s, υ_{O-H} 2908–2516 b, $\upsilon_{asym\ C=O}$ 1677 s, $\upsilon_{C=C}$ 1505 s, $\upsilon_{sym\ C=O}$ 1425, $\Delta\upsilon = \upsilon_{asym} - \upsilon_{sym}$ 255, $\upsilon_{O_2-CH_2}$ 928 s. ^{1}H NMR data: ($CDCl_3$, 293 K) δ ppm 8.15 s, 1H (*acrylic proton*), 7.19–7.41 m, 4H (*aromatic protons*), 6.28–6.32 m, 3H (*aromatic protons*), 5.94 s, 2H (*methylenedioxy protons*). ^{13}C NMR data: ($CDCl_3$, 293 K) δ ppm 171.9 (*carbonyl carbon*), 161.8 (*C–F aromatic carbon*) $^1J[^{13}C, ^{19}F]$ 225.7 Hz (147.5, 147.4, *methylenedioxy carbons*), 115.9 (*aromatic carbon*) $^2J[^{13}C, ^{19}F]$ doublet 32.3 Hz (144.0, 132.6, 131.9, 124.2, 123.3, 123.1, 121.3, 116.6, 109.6, *acrylic and aromatic carbons*), 101.4 (*methylenedioxy carbon*). Mass fragmentation, *m/z* (%): $[C_{16}H_{11}O_4F]$ 286 (100), $[C_{16}H_{11}O_4]$ 266 (7), $[C_{15}H_{11}O_2F]$ 241 (5), $[C_8H_5O_2F]$ 151 (16), $[C_{16}H_{11}OF]$ 136 (22) (Figure 3).

Biological studies

Antibacterial assay

All these synthesized compounds were tested against four bacterial strains: Gram-positive [*Bacillus subtilis* (ATCC6633) and *Staphylococcus aureus* (ATCC6538)] and Gram-negative [*Escherichia coli* (ATCC15224) and *Bordetella bronchiseptica* (ATCC4617)]. The agar well-diffusion method was used for the determination of inhibition zones[9]. Briefly, 0.75 mL of the broth culture containing ~10^6 colony-forming units per mL of the test strain was added to the 75 mL of nutrient agar medium at 45°C, mixed well, and then poured into a 14-cm sterile Petri plate. The media were allowed to solidify, and 8-mm wells were dug with a sterile metallic borer. Then a dimethyl sulphoxide (DMSO) solution of test sample (100 µL) at 1 mg/mL was added to the respective wells. DMSO served as negative control and the standard antibacterial drug ciprofloxacin (1 mg/mL) were used as positive controls. Triplicate plates of each bacterial strain were prepared. The plates were incubated aerobically at 37°C for 24 hours. The activity was determined by measuring the diameter of zone showing complete inhibition (mm). Thereby zones were precisely measured with the aid of a vernier caliper (precision ± 0.1 mm). The growth inhibition was calculated with reference to the positive control.

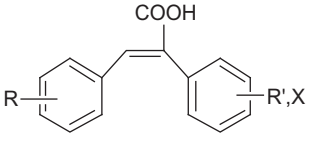


Figure 3. The structure of substituted phenyl acrylic acids.

Antifungal assay

Antifungal activity against *Saccharomyces cerevisiae* was determined by using Agar tube dilution method[9]. Screw-caped test tubes containing sabouraud dextrose agar medium (4 mL) were autoclaved at 121°C for 15 minutes. Tubes were allowed to cool to 50°C and non-solidified sabouraud dextrose agar was loaded with 66.6 µL of compound from the stock solution (12 mg/mL in DMSO) to make 200 µL/mL final concentration. Tubes were then allowed to solidify in a slanted position at room temperature. Each tube was inoculated with a 4-mm diameter piece of inoculum from a 7-day-old fungal culture. The medium supplemented with DMSO and Nystatin (200 µL/mL) was used as negative and positive controls, respectively. The tubes were incubated at 28°C for 7 days and growth was determined by measuring linear growth (mm) and growth inhibition was calculated with reference to the negative control.

Results and discussion

Infrared spectroscopy

Infrared spectroscopy is still considered a powerful tool in the area of hydrogen bond research in carboxylic acids. Of particular interest are the spectral properties of the υ_{X-H} bands in the IR, connected with the excitation of proton-stretching vibrations in the X–H. . . Y hydrogen bridges. Hydrogen bond formation in molecular systems is responsible for a strong increase of υ_{X-H} band integrated intensities, a considerable decrease of band frequencies, and their considerable widening when compared with corresponding band parameters for nonassociated X–H groups[10]. FTIR spectra of all ligands revealed that dimerization and trimerization occur mostly through carboxylic interactions. These interactions are the basis of broadening of the OH bond peak and the slight decrease in the stretching frequency of the C=O bond. The large $\Delta\upsilon$ values also verify that the carboxylic group has no strong coordination with any other group. Furthermore, major stretching frequencies like carbon–carbon double bonds and the methylenedioxy moiety[11] are also visible in their respective regions at 1511–1486 and 928 cm^{-1}, respectively. Data are given in experimental section.

^{1}H NMR spectroscopy

In the spectra of acids, doublets and multiplets in the aromatic region have been assigned to substituted phenyl moieties and acrylic protons. In such complex circumstances, the location of particular protons cannot be surely determined because of splitting due to the coupling of protons of the aromatic region with each other and with substituted groups, for example, fluoro groups. However, a significant doublet of protons appears in compounds (2), (3), (4), (5), and (6) at 7.32–7.51 ppm with coupling of 7.5–8.4 Hz. This is consistent with protons close to a trifluoromethyl group. An attempt has also been made to assign the other signals in some of the compounds. In compounds (1) and (2), a doublet of doublets because of the coupling of adjacent protons and fluorine appears at a range of 6.59–7.14 ppm. The $^{3}J[^{19}F, ^{1}H]$ coupling values observed are 7.8 and 8.7 Hz, respectively, whereas $^{3}J(^{1}H, ^{1}H)$ stands at 6.0 and 6.6 Hz, respectively. Compound (7) shows a triplet in this region while compound (8) does not. The olefinic protons, in each case, resonate as a sharp singlet at a relatively downfield region in the range of 7.33–8.19 ppm[12]. The hydroxyl proton resonance is obtained in many of the spectra but fades away in some cases because of the lability of a proton attached to a carboxylic group in solution. In compounds (7) and (8), methylenedioxy protons are located by the presence of singlet at 5.94–6.00 ppm as reported[11]. Protons of substituted groups (methyl and methoxy groups) can also be distinguished as significant singlets in the alkyl region, that is, 2.65 and 3.72 ppm, respectively.

^{13}C NMR spectroscopy

To confirm the proposed structures of the compounds, ^{13}C NMR data should be consistent with ^{1}H NMR and IR data. The characteristic resonance peaks assigned provided the expected results. In ^{13}C NMR, upfield or downfield shifts and couplings, $^{n}J[^{13}C, ^{19}F]$, of a particular carbon provides sufficient information regarding the substituents. In addition to the aromatic carbons, other carbons, for example, acrylic carbons, also show signals in this region because of a downfield shift. Coupling due to fluorine atoms either directly attached to the aromatic ring or in any other case (e.g., trifluoromethyl) also causes some difficulties in assigning carbon atoms. Carbonyl groups appear at 167.5–173.2 ppm. A strong $^{n}J[^{13}C, ^{19}F]$ coupling in compounds (1), (2), (7), and (8) results in the emergence of two peaks at 164.4–161.8 ppm having ^{1}J $[^{13}C, ^{19}F]$ values at about 227–246 Hz. A doublet due to $^{n}J[^{13}C, ^{19}F]$ coupling is observed in almost all of the cases having F and CF_3 as substituents. However, C–F coupling values in the case of trifluoromethyl, observed for[13], are nearly 250–300 Hz. In this case, the magnitude of ^{1}J[C–F], ^{2}J[C–F] coupling ranges between 25–49 Hz and 6–32.2 Hz, respectively. Carbons of the methylenedioxy group in compounds (7) and (8) resonate at 101.4 ppm and other substituted groups, for example, methyl and methoxy carbons, are also present in their specific regions.

GC–mass spectroscopy

Mass spectrometry is also an important supporting technique for determining the structure of these molecules. In many of these compounds, a uniform pattern of fragmentation and in some cases, a significant molecular ion peak is observed that confirms the structure of many of the compounds. For example, in compound (8), a molecular ion peak which is also the base peak is observed at m/z 286. The fragmentation mainly favors the loss of a carbonyl group and substituted groups whereas the loss of a hydroxyl group (OH) is less favorable[14].

X-ray crystallography

The crystal structure of compound (1) demonstrates *E*-configuration about the acrylate moiety, and symmetry-related molecules are linked by O–H. . . O bonds, making centro-symmetric carboxylic acid dimers (Figures 4 and 6). These dimers are arranged in layers that are further connected by small links which form a slab-like structure for the compound.

The distance of the central C=C confirms double bond character, which is evidence of delocalized bonding in the acrylate moiety. The bond lengths, C=O and C–O are 1.247Å and 1.299Å, respectively, show to some extent a resonance between single and double bond character. In all, the bond lengths and bond angles, similar behavior is observed[15,16], but the aromatic rings are not coplanar [14]. The dihedral angle between the aromatic ring planes is 69.5° and 66.7° for compound (1) and compound (3), respectively. This arrangement is similar to that reported earlier[16].

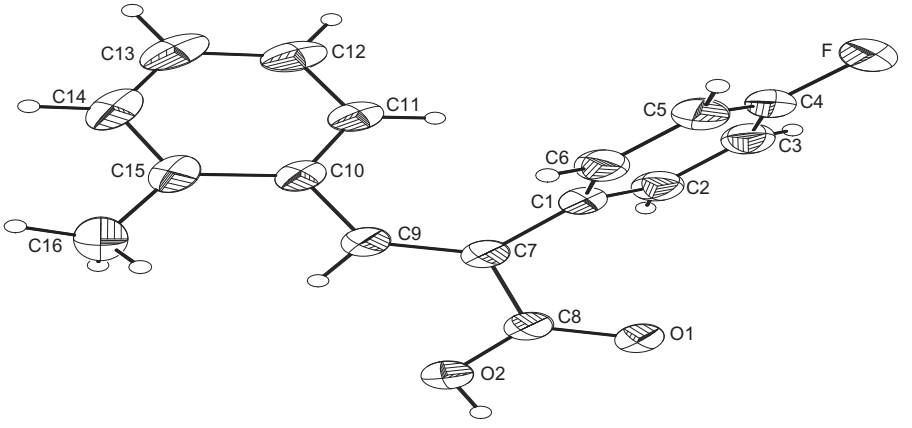


Figure 4. Crystal structure of compound (1) showing *E*-configuration.

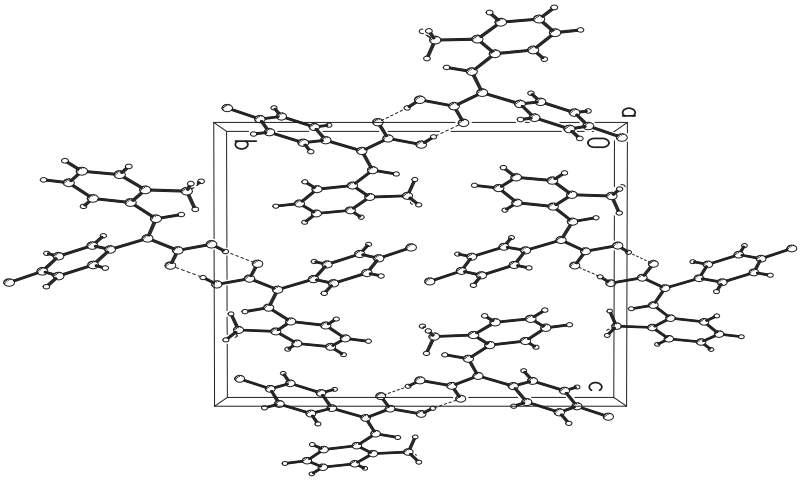


Figure 5. Closed packing of compound (1) showing hydrogen bonding.

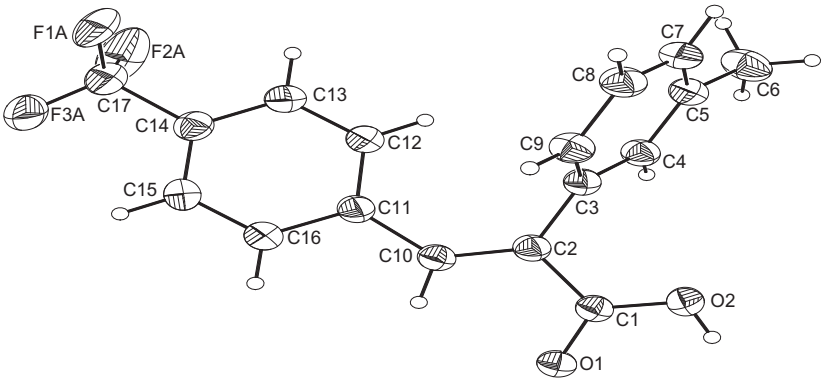


Figure 6. Crystal structure of compound (3) showing *E*-configuration.

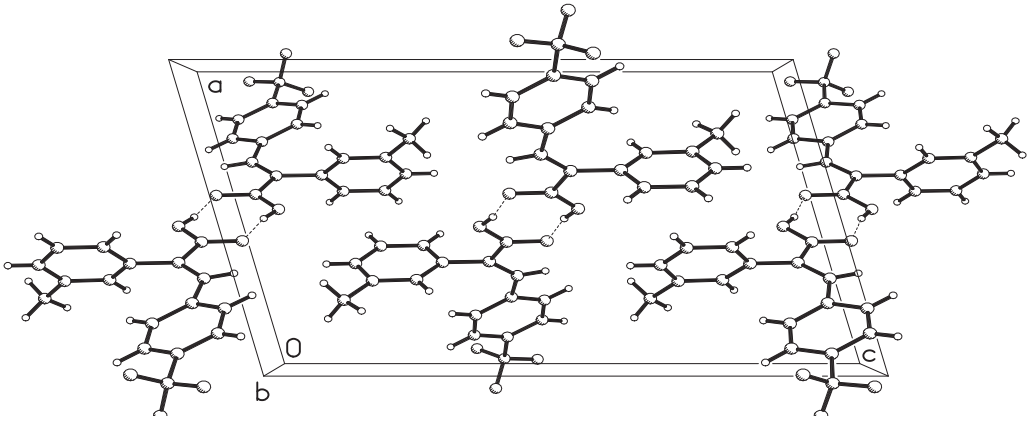


Figure 7. Closed packing of compound (3) showing hydrogen bonding.

Compound (3) exhibits almost the same behavior with *E*-stereoselectivity as observed in compound (1). All the F-C bond lengths are quite normal as discussed earlier[7,8,17]. The *E*-configuration for compound (7) has also been previously confirmed by X-ray crystallography[7,8] (Figures 5 and 7, Tables 1–5).

Biological studies

In vitro biological screening tests of synthesized compounds were carried out for antibacterial activity. The activity was tested against four bacterial strains. The agar well-diffusion method[9,18,19] was used in these assays and each experiment was performed in triplicate. Readings of the zone of inhibition represent the mean value of three readings with standard deviation, which are shown in Table 6. Criteria for activity are based on inhibition zone (mm); inhibition zone more than 12 mm shows significant activity, for 10–12 mm inhibition activity is good, 7–9 mm is low, and below 7 mm is nonsignificant activity. All bacterial strains have clinical implication; *S. aureus* causes food poisoning, scaled skin syndrome, and endocarditis; *B. subtillis* causes food poisoning; *E. coli* causes infection of wounds, urinary tract, and dysentery; and *B. bronchiseptica* causes pyrexia, sneezing, nasal discharge, and spontaneous or induced coughing. All chloro- and fluoro-substituted acid derivatives have been found active against all bacterial strains. Most probably, this is because of the development of small interaction by halo substituents. These small interactions may cause increase in hydrolysis and up to some extent lipophilicity, which are the main factors for the improvement of potency of these compounds. These compounds have also been found effective against fungal strain *Saccharomyces cerevisiae* because of their improvement of above-mentioned factors. The antibacterial and antifungal data are given in Table 6.

Table 1. Crystal data and structure refinement parameters for compounds (1) and (3).

Parameters	Compound (1)	Compound (3)
Empirical formula	$C_{16}H_{13}FO_2$	$C_{17}H_{13}F_3O_2$
Formula weight	256.26	306.27
Crystal system	Monoclinic	Monoclinic
Space group	$P2_1/c$	$P2_1/c$
Unit cell dimensions		
a (Å)	5.8883(4)	11.8892(5)
b (Å)	15.3071(9)	6.7587(3)
c (Å)	15.1413(9)	19.0474(10)
α (°)	90	90
β (°)	91.363(5)	104.116(4)
γ (°)	90	90
V(Å^3)	1364.34(15)	1484.35(12)
Z	4	4
Crystal size (mm)	$0.55 \times 0.50 \times 0.45$	$0.55 \times 0.28 \times 0.25$
F(000)	536	632
Reflections collected	5071	2766
Independent reflections	2487 [R(int) = 0.0240]	2641 [R(int) = 0.0176]
R indices (all data)	R1 = 0.0688, wR2 = 0.1889	R1 = 0.0762, wR2 = 0.2154
Final R indices [$I > 2\sigma(I)$]	R1 = 0.0630, wR2 = 0.1815	R1 = 0.0692, wR2 = 0.1994
Goodness-of-fit	1.049	1.080
Theta range for data collection (°)	4.11–69.12	3.83–68.94
Data/restraints/parameters	2487/0/175	2641/12/231

Table 2. Selected bond angles of compound (1).

Bond angle	(°)	Bond angle	(°)
O(1)–C(8)–C(7)	120.01(17)	O(2)–C(8)–C(7)	117.44(16)
C(9)–C(7)–C(8)	118.43(17)	C(8)–C(7)–C(1)	115.29(14)
C(9)–C(7)–C(1)	126.28(15)	O(1)–C(8)–O(2)	122.55(16)
C(7)–C(9)–C(10)	129.29(19)	C(11)–C(10)–C(15)	118.58(18)
C(6)–C(1)–C(2)	118.83(17)	C(6)–C(1)–C(7)	120.07(15)
C(2)–C(1)–C(7)	121.05(16)	C(11)–C(10)–C(9)	122.47(18)

Table 3. Selected bond lengths of compound (1).

Bond length	(Å)	Bond length	(Å)
F–C(4)	1.364(2)	O(1)–C(8)	1.247(2)
O(2)–C(8)	1.299(2)	C(1)–C(6)	1.381(3)
C(1)–C(2)	1.383(2)	C(10)–C(11)	1.390(3)
C(10)–C(15)	1.402(3)	C(7)–C(9)	1.343(2)
C(7)–C(8)	1.478(2)	C(9)–C(10)	1.469(2)
C(1)–C(7)	1.487(2)	C(9)–H(9A)	0.9300

Table 4. Selected bond angles for compound (3).

Bond angle	(°)	Bond angle	(°)
C(10)–C(2)–C(1)	116.80(19)	C(3)–C(2)–C(1)	116.65(19)
C(9)–C(3)–C(4)	118.6(2)	C(9)–C(3)–C(2)	120.9(2)
C(4)–C(3)–C(2)	120.5(2)	C(16)–C(11)–C(12)	117.7(2)
C(16)–C(11)–C(10)	117.2(2)	C(12)–C(11)–C(10)	125.1(2)
F(2A)–C(17)–F(1B)	97.6(5)	F(2A)–C(17)–F(3A)	109.10(12)
C(10)–C(2)–C(3)	126.5(2)	C(12)–C(11)–C(10)	125.1(2)

Table 5. Selected bond lengths for compound (3).

Bond length	(Å)	Bond length	(Å)
O(1)–C(1)	1.269(3)	O(2)–C(1)	1.264(3)
F(1A)–C(17)	1.2903(19)	C(1)–C(2)	1.487(3)
C(2)–C(10)	1.340(3)	C(10)–C(11)	1.469(3)
C(2)–C(3)	1.487(3)	C(5)–C(6)	1.503(4)
C(11)–C(16)	1.386(3)	C(11)–C(12)	1.393(3)
C(3)–C(9)	1.384(3)	C(3)–C(4)	1.387(3)

Conclusion

In this article, we have reported the synthesis of eight phenyl acrylic acids, and their structure has been studied in solid and solution state by using instrumental techniques like, FTIR spectroscopy, multinuclear (^{1}H, ^{13}C) NMR spectroscopy, and GC-MS. In the solid state, X-ray crystal structures of the title compounds show strong intermolecular hydrogen bonding, which leads to the formation of dimers. These compounds have also been tested against four bacterial strains and one fungal strain and has shown significant activity against drug-resistant bacterial strains.

Table 6. Antibacterial and antifungal activity of selected compounds.

	Mean zone of inhibition (mm)				% Growth inhibition
Compound no.	*S. aureus*	*B. bronchiseptica*	*E. coli*	*B. subtilis*	*S. cervevisiae*
(1)	14.5 ± 0.23	15.0 ± 0.14	13.0 ± 0.14	14.0 ± 0.20	15.0 ± 0.90
(2)	18.2 ± 0.45	18.0 ± 0.25	18.2 ± 0.19	18.0 ± 0.19	17.4 ± 1.20
(3)	20.0 ± 0.20	19.6 ± 0.21	19.8 ± 0.14	19.8 ± 0.09	17.0 ± 081
(4)	20.5 ± 0.10	20.0 ± 0.24	20.3 ± 0.18	20.2 ± 0.07	17.5 ± 0.65
(5)	17.0 ± 0.15	18.0 ± 0.08	16.6 ± 0.21	17.0 ± 0.21	16.8 ± 1.10
(6)	20.3 ± 0.35	19.8 ± 0.10	20.2 ± 0.20	20.0 ± 0.16	17.2 ± 1.45
(7)	19.0 ± 0.09	18.0 ± 0.22	17.6 ± 0.24	17.0 ± 0.09	18.0 ± 1.37
(8)	20.0 ± 0.21	19.6 ± 0.15	18.0 ± 0.25	17.0 ± 0.21	19.0 ± 0.95
Ciprofloxacin	30.4 ± 0.50	30.0 ± 0.21	30.2 ± 0.50	30.2 ± 0.43	—
Nystatin	—	—	—	—	22.5 ± 0.65
DMSO	—	—	—	—	—

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

Supplementary materials

Crystallographic data for the structural analysis have been deposited with Cambridge Crystallographic Data Centre, CCDC Nos. 689445 and 689446 for compounds (1) and (3), respectively. Copies of information may be obtained free from the Director, CCDC, 12 Union Road, Cambridge, CBZIEZ, UK. E-mail:deposit@ccdc.cam.ac.uk or http://www.ccdc.cam.ac.uk.

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