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Der Pharma Chemica, 2012, 4(4):1445-1457
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Synthesis, Characterization and Biological Evaluation of Functionalized Derivatives of Versatile Synthon 4,4'-Difluoro chalcone

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ABSTRACT

Some functionalized fluorinated derivatives are prepared by condensing 4,4'-difluoro chalcone with hydrazine derivatives, o-phenylenediamine, ammonium acetate/acetic acid, ethyl cyanoacetate, malononitrile, acetylacetone, esters of acetoacetic acid, acetoacetanilde, aminoguanidine hydrochloride, urea and thiourea. All these derivatives are characterized by NMR, IR, mass spectral and also some of them by single crystal XRD data. All the synthesized products are screened for their in vitro antimicrobial and antioxidant properties. Majority of the tested compounds exhibited significant antimicrobial activity and some of them showed DPPH scavenging activity.

Keywords: Synthesis, Characterization, Antimicrobial, DPPH scavenging assay, 4,4'-difluoro chalcone.

INTRODUCTION

The introduction of fluorine substituents into an organic molecule can readily change the physicochemical properties of the molecule. Including fluorine atom in potential medicines can have a variety of dramatic effect on the molecule's properties, perhaps making them more selective, increasing their efficacy, or making them easier to administer [1]. There are very few naturally occurring organic compounds that contain fluorine, but all the known naturally occurring fluoro-organic compounds are poisonous [2]. The finding of fluorine in teeth enamel, ash of blood, and the yolk and shell of eggs stimulated the search for its physiological role [3,4]. So, today more than one million compounds containing one or more carbon-fluorine bonds are known.

Many fluorinated compounds are widely used as antidepressants, anti-inflammatory agents, antimalarial drugs, antipsychotics, antiviral agents, and general anaesthetics [5]. Examples of selectively fluorinated drugs include Midazolam (general anesthetics), Progabide (antidepressants), Paroxetine (antidepressants), Ezetimibe (antilipemics), Atorvastatin (antilipemics) and Linezolid (antibiotics).

One of the most important factors in drug design is that fluorine is much more lipophilic than hydrogen, so incorporating fluorine atom in a molecule will make it more fat soluble. This means it percolates into membranes much more readily, and hence the fluorinated molecule has a higher bioavailability [6,7]. So it is no great surprise that around a fifth of all drugs on the market today contain at least one fluorine substituent. The inclusion of a fluorine atom in a drug molecule can influence not only pharmacokinetic properties, such as absorption, tissue distribution, secretion, and the route and rate of biotransformation but also its pharmacodynamics and toxicology [8]. Introducing F and CF_3 substituents often improves lipophilicity, and suppresses metabolic detoxification processes to increase the *in vivo* lifetime of drugs [9].

Organofluorine chemistry is virtually a completely man-made branch of organic chemistry. Since none of the natural C-F compounds are isolated for utilization, synthetic routes to a wide variety of fluoro-organic molecules have been developed, and an impressive array of reagents exists for creating a C-F bond (the strongest single bond involving carbon). Elemental fluorine is generally too reactive for use in direct reactions with organic compounds, but favorable results could be achieved through the choice of specific reaction conditions (degree of dilution, temperature, the nature of solvent). Drug designers frequently use naturally occurring molecules as starting points in their studies, altering substitution patterns to change its properties to make it more effective, more selective, or both [10].

Fluorine and its chemistry often have been described by the adjectives i.e. exciting, exotic, unusual, unexpected, novel, highly reactive and challenging. In view of the importance of organofluorine compounds, it was decided to prepare different derivatives of 4,4'-difluoro chalcone by condensing it with hydrazine derivatives, o-phenylenediamine, ammonium acetate/acetic acid, ethyl cyanoacetate, malononitrile, acetylacetone, esters of acetoacetic acid, acetoacetanilde, aminoguanidine hydrochloride, urea and thiourea, and to study their biological activities.

MATERIALS AND METHODS

2.1. Chemistry

Melting points were taken in open capillary tubes and are uncorrected. The purity of the compounds was confirmed by thin layer chromatography using Merck silica gel 60 F_{254} coated aluminium plates. IR spectra were recorded on Shimadzu-FTIR Infrared spectrometer in KBr (ν_{max} in cm^{-1}). ^{1}HNMR (400 MHz) spectra were recorded on a Bruker AMX 400 spectrometer, with 5mm PABBO BB -1H TUBES and ^{13}C (100 MHz) NMR spectra were recorded for approximately 0.03 M solutions in DMSO-d_6 at 100MHz with TMS as internal standard. LCMS were obtained using Agilent 1200 series LC and Micromass zQ spectrometer. Elemental analysis was carried out by using VARIO EL-III (Elementar Analysensysteme GmBH).

General procedure for synthesis of (2*E*)-1,3-bis(4-fluorophenyl)prop-2-en-1-one (1)

To a mixture of 4-fluoroacetophenone (13.8 g, 0.1 mol) and 4- fluorobenzaldehyde (12.4 g, 0.1 mol) in 30mL ethanol, 10 mL of 10 % sodium hydroxide solution was added and stirred at 5-10ºC for 3 h. The precipitate formed was collected by filtration and recrystallized in ethanol to get off white crystals. Yield 91%; mp 113-115 ºC. IR (KBr, ν_{max} in cm^{-1}): 1662 (C=O), 1600(C=C), 1220 (C-F). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.73(d, 1H, $H_{\alpha,}$ J= 15.6 Hz), 7.90 (d, 1H, $H_{\beta,}$ J = 15.6Hz), 7.28-8.27(m, 8H, ArH). LCMS: m/z 244 (M^+). Anal. Calcd. for $C_{15}H_{10}F_2O$: C, 73.76; H, 4.13. Found: C, 73.70; H, 4.09%.

Procedure for synthesis of 3,5-bis(4-fluorophenyl)-1-aryl-4,5-dihydro-1*H*-pyrazole (2a, b)

A mixture of 4,4'-difluoro chalcone (2.44 g, 0.01 mol) and 4-nitro phenyl hydrazine (1.53 g, 0.01 mol) or 2,4-dinitrophenylhydrazine (1.98 g, 0.01 mol) in glacial acetic acid (20 mL) was refluxed for 8 h. The reaction mixture was cooled and poured into ice-cold water (50 mL). The precipitate was collected by filtration and purified by recrystallization from ethanol.

3,5-Bis(4-fluorophenyl)-1-(4-nitrophenyl)-4,5-dihydro-1H-pyrazole **(2a)**

Yellow crystals. Yield: 74%; mp 167-170ºC. IR (KBr, ν_{max} in cm^{-1}); 2922 (CH_2), 1597 (C=N), 1504 (C-NO_2), 1217 (C-F). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.24 (dd, 1H, $H_{A,}$ J_{AB}= 18.06 Hz, J_{AX} = 4.00 Hz), 3.97 (dd, 1H, $H_{B,}$ J_{AB}= 17.93 Hz, J_{BX} =11.97 Hz), 5.75 (dd, 1H, H_X, J_{XA} = 4.00Hz, J_{XB} = 11.75Hz), 7.07-8.07 (m, 12H, Ar-H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 43.16 (CH_2), 61.65 (CH), 111.94, 115.72 (t), 125.71, 127.77 (d), 128.73 (d), 137.15,

138.08, 148.08, 151.69, 160.33, 161.82, 162.76, 164.29. LCMS: m/z 379.9 (M^++1). Anal. Calcd. for $C_{21}H_{15}F_2N_3O_2$: C, 66.49; H, 3.99; N, 11.08. Found: C, 66.44; H, 4.01; N, 11.05%.

1-(2,4-Dinitrophenyl)-3,5-bis(4-fluorophenyl)-4,5-dihydro-1H-pyrazole **(2b)**
Red crystals. Yield: 60%; mp 190-192ºC. IR (ν_{max} in cm^{-1}); 1603 (C=N), 1504 (C-NO_2), 1215 (C-F). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.14 (dd, 1H, H_A, J_{AB} = 17.52 Hz, J_{AX} = 6.34 Hz), 3.86 (dd, 1H, H_B, J_{AB}= 17.54 Hz, J_{BX} =12.20 Hz), 5.48 (dd, 1H, H_X, J_{AX} = 6.34 Hz, J_{BX} = 12.18 Hz), 6.70-7.80 (m, 11H, Ar-H). LCMS: m/z 424.3 (M^+). Anal. Calcd. for $C_{21}H_{14}F_2N_4O_4$: C, 59.44; H, 3.33; N, 13.20. Found: C, 59.41; H, 3.32; N, 13.17%.

Procedure for synthesis of 3,5-bis(4-fluorophenyl)-4,5-dihydro-1*H*-pyrazoles (3a-c)
A mixture of 4,4'-difluoro chalcone (2.44 g, 0.01 mol) and hydrazine hydrate (0.5 mL, 0.01 mol) in 20 mL formic acid/ acetic acid/ butyric acid was refluxed for 8 h. The reaction mixture was cooled and poured into 50 mL of ice-cold water. The precipitate was collected by filtration and purified by recrystallization from ethanol.

3,5-Bis(4-fluorophenyl)-4,5-dihydro-1H-pyrazole-1-carbaldehyde **(3a)**
Colourless crystals. Yield: 86%; mp 133-135ºC. IR (KBr, ν_{max} in cm^{-1}): 2927 (CH_2), 1649 (CHO), 1602 (C=N), 1228 (C-F). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.19 (dd, 1H, H_A, J_{AB} = 18.0 Hz, J_{AX} = 5.0 Hz), 3.86 (dd, 1H, H_B, J_{AB}= 18.0 Hz, J_{BX} =12.0 Hz), 5.52 (dd, 1H, H_X, J_{AX} = 5.0 Hz, J_{BX} = 12.0 Hz), 7.14-7.86 (m, 8H, Ar-H), 8.88 (s, 1H, CHO). LCMS: m/z 286.3 (M^+). Anal. Calcd. for $C_{16}H_{12}F_2N_2O$: C, 67.13; H, 4.23; N, 13.27. Found: C, 67.10; H, 4.25; N, 13.21%.

1-[3,5-Bis(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-1-yl]ethanone **(3b)**
Colourless crystals. Yield: 84%; mp 112-114ºC. IR (KBr, ν_{max} in cm^{-1}): 2926 (CH_2, CH_3), 1645 (C=O), 1602 (C=N), 1224 (C-F). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.37 (s, 3H, $COCH_3$), 3.11 (dd, 1H, H_A, J_{AB} = 18.09 Hz, J_{AX} = 4.66 Hz), 3.81 (dd, 1H, H_B, J_{AB}= 18.05 Hz, J_{BX} =11.72 Hz), 5.54 (dd, 1H, H_X, J_{AX} = 4.65 Hz, J_{BX} = 11.70 Hz), 7.01-7.86 (m, 8H, Ar-H). LCMS: m/z 300.9 (M^++1). Anal. Calcd. for $C_{17}H_{14}F_2N_2O$: C, 67.99; H, 4.70; N, 9.33. Found: C, 67.86; H, 4.62; N, 9.29 %.

1-[3,5-Bis(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-1-yl]butan-1-one **(3c)**
Off white crystals. Yield: 76%; mp 113-115ºC. IR (KBr, ν_{max} in cm^{-1}); 2966, 2931(CH_2, CH_3), 1658 (C=O), 1602 (C=N), 1226 (C-F). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 0.87 (t, 3H, CH_3), 1.53 (m, 2H, $COCH_2$), 2.59 (m, 2H, CH_2), 3.10 (dd, 1H, H_A, J_{AB} = 18.15 Hz, J_{AX} = 4.75 Hz), 3.79 (dd, 1H, H_B, J_{AB}= 18.15 Hz, J_{BX} =11.87 Hz), 5.52 (dd, 1H, H_X, J_{XA} = 4.70 Hz, J_{XB} = 11.83 Hz), 7.11-7.85 (m, 8H, Ar-H). LCMS: m/z 328.0 (M^+). Anal. Calcd. for $C_{19}H_{18}F_2N_2O$: C, 69.50; H, 5.53; N, 8.53. Found: C, 69.51; H, 5.53; N, 8.49%.

Procedure for synthesis of 4,6-bis(4-fluorophenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (4)
A mixture of 4,4'-difluoro chalcone (2.44 g, 0.01 mol), ethyl cyanoacetate (1.13 g, 0.01 mol) and ammonium acetate (1.54 g, 0.02 mol) in ethanol (20 mL) was refluxed for 8 h. The reaction mixture was cooled and poured into ice-cold water (50 mL). The precipitate was collected by filtration and recrystallized in ethanol to get off white powder. Yield: 68%; mp 265-268ºC. IR (KBr, ν_{max} in cm^{-1}): 3456 (NH), 2220 (CN),1649 (C=O), 1232 (C-F). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.33-8.41(m, 9H, Ar-H), 12.82(s, 1H, NH). LCMS: m/z 309.3 (M^++1). Anal. Calcd. for $C_{18}H_{10}F_2N_2O$: C, 70.13; H, 3.27; N, 9.09. Found: C, 70.10; H, 3.26; N, 9.07%.

Procedure for synthesis of 2-amino-4,6-bis (4-fluorophenyl) pyridine-3-carbonitrile (5)
A mixture of 4,4'-difluoro chalcone (2.44 g. 0.01 mol), malononitrile (0.66 g, 0.01 mol) and ammonium acetate (1.54 g, 0.02 mol) in ethanol (20 mL) was refluxed for 10 h. The reaction mixture was cooled and poured into ice-cold water (50 mL). The precipitate was collected by filtration and recrystallized in ethanol to get off white powder. Yield: 73%; mp 204-206ºC. IR (KBr, ν_{max} in cm^{-1}): 3500, 3396 (NH), 2206 (CN),1610 (C=N), 1224 (C-F). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.27-8.20(m, 9H, Ar-H), 7.01 (s, 2H, NH_2). LCMS: m/z 307.9 (M^++1). Anal. Calcd. for $C_{18}H_{11}F_2N_3$: C, 70.35; H, 3.61; N, 13.67. Found: C, 70.32; H, 3.59; N, 13.64%.

Procedure for synthesis of 2,4,6-tris(4-fluorophenyl)pyridine (6)
A mixture of 4,4'-difluoro chalcone (2.44 g, 0.01 mol) and ammonium acetate (1.54 g, 0.02mol) in glacial acetic acid (10 mL) was refluxed for 6 h. The reaction mixture was cooled and poured into ice-cold water (50 mL). The

precipitate was collected by filtration and recrystallized in ethanol to get white powder. Yield: 56%; mp 205-207ºC (Reported mp 205ºC). Anal. Calcd. for $C_{23}H_{14}F_3N$: C, 76.45; H, 3.91; N, 3.88. Found: C, 76.41; H, 3.92; N, 3.86%.

Procedure for synthesis of 2,4-bis(4-fluorophenyl)-2,3-dihydro-1*H*-1,5-benzodiazepine (7)
In the absence of sunlight, a solution of 4,4'-difluoro chalcone (2.44g, 0.01mol) and 1, 2-diaminobenzene (1.08g, 0.01mol) in absolute ethanol (15 mL) was refluxed in the presence of triethyl amine (3 mL) for 15 h. The reaction mixture was cooled to 0 °C and left overnight. The precipitate formed was filtered off and recrystallized from ethanol affording light yellow crystals**.** Yield: 74%; mp 133-136ºC. IR (KBr, ν_{max} in cm^{-1}): 3350 (NH), 3045, 2856 (CH_2), 1921, 1600 (C=N), 1226 (C-F). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.00-3.05 (dd, 1H, CH_2-H_A, J_{AX} = 3.68Hz, J_{AB} = 13.73 Hz), 3.07-3.13 (dd, 1H, CH_2-H_B, J_{BX}= 6.92 Hz, J_{BA} = 13.73Hz), 5.22-5.24 (t, 1H, CH-H_x , J_{XA} = 3.2Hz, J_{XB} = 6.32Hz), 5.88(s, 1H, NH), 6.83-7.80(m, 12H, Ar-H). ^{13}C NMR (DMSO-d_6, 100 MHz); 37.86 (CH_2), 67.88 (CH), 115.19 (d), 115.40 (d), 119.80, 120.65, 126.78, 128.49 (d), 129.45 (t), 136.03 (d), 137.76, 140.48, 141.87 (d), 160.53, 162.46, 162.95, 164.92 (d). LCMS: m/z 334.9 (M^++1). Anal. Calcd. for $C_{21}H_{16}F_2N_2$: C, 75.43; H, 4.82; N, 8.38. Found: C, 75.39; H, 4.83; N, 8.34%.

Procedure for synthesis of alkyl 4,6-bis(4-fluorophenyl)-2-oxocyclohex-3-ene-1-carboxylate (8a, b)
A mixture of 4,4'-difluoro chalcone (2.44 g, 0.01mol) and methyl acetoacetate/ ethyl acetoacetate (0.01 mol) in ethanol was refluxed for 4 h in 10-15 mL of ethanol in the presence of 0.8 mL 10% NaOH. The reaction mixture was cooled to room temperature and the solid product obtained was filtered and recrystallized from ethanol.

Methyl 4,6-bis(4-fluorophenyl)-2-oxocyclohex-3-ene-1-carboxylate **(8a)**
Off white crystals. Yield: 78%; mp 140-144 ºC. IR (KBr, ν_{max} in cm^{-1}): 2951, 2897 (CH_2, CH_3), 1660 (C=O ketone), 1739 (C=O ester), 1234 (C-F). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.07(dd, 1H, H_A), 3.10 (d, 1H, $CHCO_2Me$), 3.53 (s, 3H, OCH_3), 3.71 (m, 1H, Ar-CH), 3.93 (m, 1H, H_B), 6.49 (s, 1H, C=CH), 7.04-7.73 (m, 8H, Ar-H). LCMS: m/z 342.9 (M^++1). Anal. Calcd. for $C_{20}H_{16}F_2O_3$: C, 70.17; H, 4.71. Found: C, 70.14; H, 4.69%.

Ethyl 4,6-bis(4-fluorophenyl)-2-oxocyclohex-3-ene-1-carboxylate **(8b)**
Off white crystals. Yield: 85%; mp 104-106ºC IR (KBr, ν_{max} in cm^{-1}): 2987, 2902 (CH_2, CH_3), 1668 (C=O ketone), 1739 (C=O ester), 1226 (C-F). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 0.90-0.93(t, 3H, CH_3); 3.00- 3.13(m, 1H, H_α), 2.96-2.97 (d, 1H, $CHCO_2Et$), 3.62-3.70 (m, 1H, Ar-CH), 3.89-3.94 (q, 2H, OCH_2), 4.07-4.16 (m, 1H, H_β), 6.54 (s, 1H, C=CH), 7.14-7.81 (m, 8H, Ar-H). LCMS: m/z 356.9 (M^++1). Anal. Calcd. for $C_{21}H_{18}F_2O_3$: C, 70.78; H, 5.09. Found: C, 70.71; H, 5.07%.

Procedure for synthesis of 4,6-bis(4-fluorophenyl)-2-oxo-*N*-phenylcyclohex-3-ene-1-carboxamide (9)
A mixture of 4,4'-difluoro chalcone (2.44 g, 0.01mol) and acetoacetanilide (1.77 g, 0.01 mol) in ethanol was refluxed for 8 h in 25 mL of ethanol in the presence of 0.8 mL 10% NaOH. The reaction mixture was cooled to room temperature and the solid product obtained was filtered and recrystallized from ethanol to get white crystals. Yield: 68%; mp 199-201ºC. IR (KBr, ν_{max} in cm^{-1}): 3425 (NH), 3076, 2926 (CH_2, CH_3), 1637 (C=O ketone), 1230 (C-F). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.08(dd, 1H, H_A), 3.03 (d, 1H, COCH), 3.82 (dd, 1H, H_B), 3.86 (t, 1H, Ar-CH), 6.56 (s, 1H, C=CH), 6.97-7.83 (m, 13H, Ar-H) 9.99(s, 1H, NH). LCMS: m/z 405.5 (M^++2). Anal. Calcd. for $C_{20}H_{16}F_2O_3$: C, 74.43; H, 4.75; N, 3.47. Found: C, 74.40; H, 4.71; N, 3.45%.

Procedure for synthesis of (6Z)-3,5-bis(4-fluorophenyl)-6-(1-hydroxyethylidene)cyclohex-2-en-1-one (10)
A mixture of 4,4'-difluoro chalcone (2.44 g, 0.01mol) and acetylacetone (1.00 g, 0.01 mol) in ethanol (25 mL) was refluxed for 6 h in the presence of 0.4 mL of 10% NaOH. The reaction mixture was cooled to room temperature and the solid product obtained was filtered and recrystallized from ethanol to get yellow crystals. Yield: 68%; mp 108-110 ºC. IR (KBr, ν_{max} in cm^{-1}): 3431 (OH), 2893(CH), 1624 (C=O), 1224 (C-F). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.96 (s, 3H, CH_3), 2.01 (s, 1H, OH), 2.95(dd, 1H, H_A), 3.25 (dd, 1H, H_B), 4.27 (t, 1H, CH), 6.56 (s, 1H, CH), 7.05-7.62 (m, 8H, Ar-H). LCMS: m/z 326.9 (M^++1). Anal. Calcd. for $C_{20}H_{16}F_2O_2$: C, 73.61; H, 4.94. Found: C, 73.58; H, 4.92%.

Procedure for synthesis of 2-[3,5-bis(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-1-yl]-4,6-bis(4-fluoro phenyl)pyrimidine (11)
A mixture of 4,4'-difluoro chalcone (2.44g, 0.01mol) and aminoguanidine hydrochloride (0.65 g, 0.005 mol) in 25 mL ethanol was refluxed for 24 h in the presence of sodium ethoxide (2 mL). The reaction mixture was cooled to

room temperature and refrigerated overnight. The solid product obtained was filtered and recrystallized from ethanol to get yellow coloured powder. Yield: 49%; mp 272-275 ºC. IR (KBr, ν_{max} in cm^{-1}): 3051 (Ar-H), 2951 (CH), 1602, 1506 (Ar C=C), 1228 (C-F). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.93 (dd, 2H, Pyrazoline CH_2), 5.91 (dd, 1H, Pyrazoline CH), 7.13-8.28 (m, 17H, Ar-H). LCMS: m/z 524 (M^+). Anal. Calcd. for $C_{31}H_{20}F_4N_4$: C, 70.99; H, 3.84; N, 10.68. Found: C, 70.95; H, 3.86; N, 10.65%.

Procedure for synthesis of 4,6-bis(4-fluorophenyl)pyrimidin-2-ol (12)

A mixture of 4,4'-difluoro chalcone (2.44 g, 0.01mol) and urea (0.60 g, 0.01 mol) in ethanol was refluxed for 18 h in the presence of sodium ethoxide (2mL). The reaction mixture was cooled to room temperature and refrigerated overnight. The solid product obtained was filtered and recrystallized from ethanol to get off white coloured powder. Yield: 54%; mp 220-222 ºC. IR (KBr, ν_{max} in cm^{-1}): 3224 (OH), 1604 (C=N), 1228 (C-F). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.04-8.27 (m, 9H, Ar-H), 11.89 (s, 1H, OH). LCMS: m/z 284.0 (M^+). Anal. Calcd. for $C_{18}H_{10}F_2N_2O$: C, 67.60; H, 3.55; N, 9.85. Found: C, 67.56; H, 3.56; N, 9.83%.

Procedure for synthesis of 2,2'-disulfanediylbis[4,6-(4-fluorophenyl)pyrimidine] (13)

A mixture of 4,4'-difluoro chalcone (2.44 g, 0.01mol) and thiourea (0.76 g, 0.01 mol) in ethanol was refluxed for 22 h in 25 mL of ethanolic KOH solution. The reaction mixture was cooled to room temperature and kept overnight. The solid product obtained on acidification with acetic acid was filtered and recrystallized from ethanol to get yellow crystalline solid. Yield: 51%; mp 198-200ºC. IR (KBr, ν_{max} in cm^{-1}): 1597 (C=N), 1228 (C-F). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.29-8.43 (m, 18H, Ar-H). LCMS: m/z 598.7 (M^++1). Anal. Calcd. for $C_{32}H_{18}F_4N_4S_2$: C, 64.20; H, 3.03; N, 9.36. Found: C, 64.14; H, 3.01; N, 9.32%.

2.2. Biological evaluation

2.2.1. Antimicrobial activity

The antimicrobial activity of synthesized compounds was carried out using agar well diffusion method. The bacterial strains were collected from different infectious status of patients who had not administered any antibacterial drugs for at least 2 weeks with the suggestions of an authorized physician, in Kiran diagnostic health centre of Chitradurga, Karnataka state, India. Fungal strains were procured from the culture maintained at National College of Pharmacy, Shimoga. The *in vitro* antimicrobial activity was carried out against 24 h culture of four bacterial strains Gram positive *Bacillus subtilis*, *Streptococcus haemolytius* Gram negative**,** *Pseudomonas aeruginosa*, *Klebsiella pneumoniae.* Two fungal strains were *Aspergillus niger and Candida albicans*. The compounds were tested at 40 µg/mL concentration against both bacterial and fungal strains. DMSO was used as a vehicle. Ciprofloxacin and Fluconazole were used as standard drugs for comparison of antibacterial and antifungal activities respectively. The zone of inhibition was compared with standard drug after 24 h of incubation at 37^{o}C for antibacterial activity and 72 h at 25^{o}C for antifungal activity.

Table 1: Antibacterial activity of the synthesized compounds

Compound	Zone of inhibition in mm (MIC in µg/µL)			
	Antibacterial strains			
	Bacillus subtilis	***Streptococcus haemolytius***	***Pseudomonas aeruginosa***	***Klebsiella pneumoniae***
1	22 (40)	21 (30)	22 (30)	21 (30)
2a	20 (30)	21 (30)	19 (30)	19 (30)
2b	22 (40)	21 (30)	20 (40)	21 (40)
3a	18 (40)	19 (30)	20 (30)	19 (30)
3b	20 (30)	20 (30)	20 (30)	20 (30)
3c	20 (30)	18 (30)	19 (30)	19 (30)
4	21 (40)	21 (30)	21 (30)	21 (30)
5	19 (30)	19 (30)	19 (30)	20 (30)
7	22 (40)	22 (40)	22 (30)	22 (40)
8a	17 (30)	18 (30)	18 (30)	18 (40)
8b	20 (30)	22 (40)	20 (20)	19 (20)
9	17 (20)	18 (30)	18 (30)	17 (30)
10	22 (30)	22 (30)	22 (30)	22 (30)
11	22 (30)	22 (30)	20 (30)	21 (30)
12	20 (40)	19 (40)	20 (30)	20 (40)
13	19 (40)	19 (30)	19 (40)	18 (40)
Control	0	0	0	0
Ciprofloxacin	23 (20)	23 (30)	22 (20)	22 (20)

Table 2: Antifungal activity of the synthesized compounds.

Compound	Zone of inhibition in mm (MIC in µg/µL)	
	Antifungal strains	
	Aspergillus niger	*Candida albicans*
1	18 (30)	20 (30)
2a	19 (30)	19 (30)
2b	22 (30)	22 (30)
3a	20 (30)	20 (30)
3b	20 (30)	20 (30)
3c	20 (30)	19 (30)
4	20 (30)	20 (30)
5	19 (30)	19 (30)
7	22 (40)	22 (40)
8a	18 (30)	18 (30)
8b	20 (30)	18 (30)
9	17 (40)	18 (40)
10	22 (40)	22 (40)
11	21 (30)	20 (30)
12	20 (40)	19 (40)
13	19 (30)	19 (30)
Control	0	0
Fluconazole	22 (30)	23 (20)

The MIC of all synthesized compounds was determined by a micro dilution method. The respective clinical strain was spread separately on the medium. The wells were created using a stainless steel sterilized cork borer under aseptic conditions. The synthesized compounds at different concentrations viz. 10, 20, 30, 40 and 50 µg was dissolved in DMSO and later loaded into corresponding wells. The zone of inhibition was compared with standard drug after 24 h of incubation at 37 ^{o}C for antibacterial activity and 72 h at 25^{o}C for antifungal activity. The results are recorded in Table **1** and **2.**

Table 3: DPPH Radical scavenging assay of synthesized compounds

Compound	% DPPH scavenging
1	4.46 ± 0.12
2a	44.85 ± 0.32
2b	51.23 ± 0.45
3a	19.98 ± 0.21
3b	17.76 ± 0.16
3c	27.30 ± 0.34
4	16.28 ± 0.11
5	28.57 ± 0.31
7	59.29 ± 0.24
8a	16.14 ± 0.29
8b	17.14 ± 0.22
9	21.66 ± 0.32
10	36.56 ± 0.37
11	20.58 ± 0.15
12	78.10 ± 0.42
13	56.42 ± 0.32
Ascorbic acid	94.56 ± 0.34

2.2.2. DPPH radical scavenging assay

The DPPH assay was based on the reported method [35]. Briefly, the DMSO sample of compounds at 50 µg/mL and it was diluted to 4 mL using methanol. To this 1mL of 1,1-diphenyl-2-picryl-hydrazyl (DPPH) solution in methanol was added. The mixed solution was incubated at room temperature for 30 min. The absorbance of stable DPPH was read at 517 nm using UV-Visible spectrophotometer and the remaining DPPH was calculated. Ascorbic acid (50µg/mL) was taken as standard. The free radical scavenging activity was expressed as follows:

$$\text{DPPH scavenging activity (\%)} = \frac{[Ac-As]}{[Ac-Ab]} \text{ X } 100$$

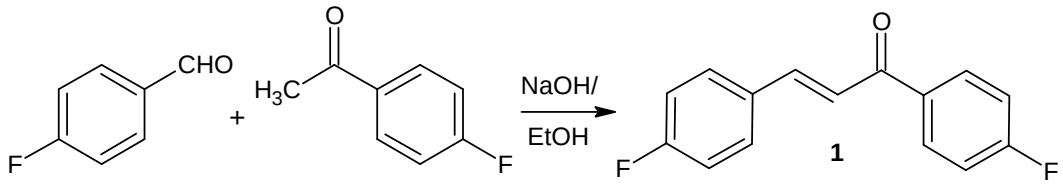
Where *Ac* was the absorbance of the control, *As* for the sample and *Ab* for the blank (MeOH+DMSO). Each sample was assayed at 50 µg/mL and all experiments were carried out in triplicate and the % radical scavenging activity is shown in Table **3.**

RESULTS AND DISCUSSION

3.1. Chemistry

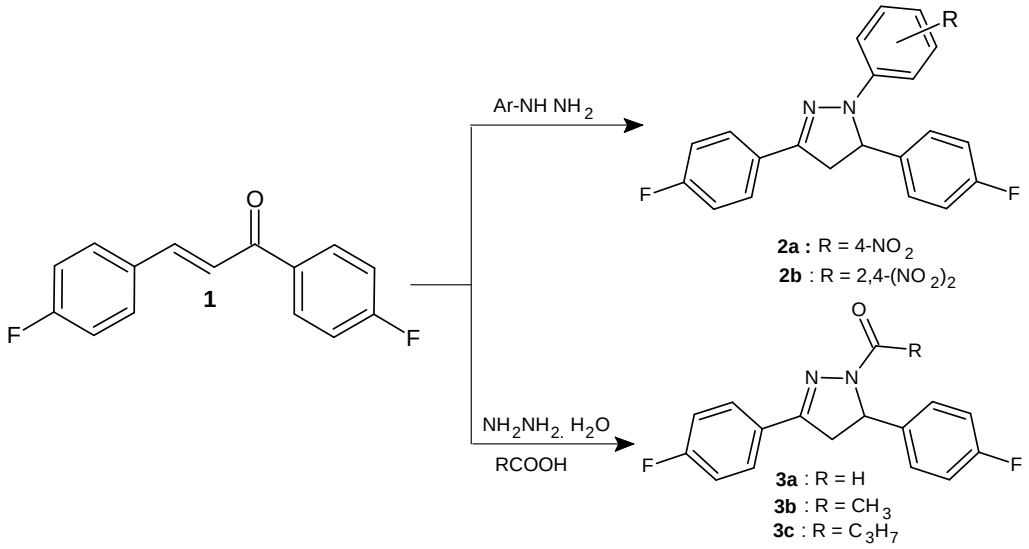
Chalcones possess high reactivity due to the presence of the carbonyl group conjugated with the double bond. This instance suggests that the nucleophiles can react with chalcones at both the carbonyl group and the double bond. The reactions with binucleophiles leading to the broad range of cyclized compounds are of particular interest [11]. The synthesis of 4,4'-difluoro chalcone and its various derivatives were accomplished according to the reaction sequences illustrated in Scheme **1-6**.

4,4′-Difluoro chalcone **1** was synthesized by the base-catalyzed Claisen– Schmidt condensation of 4-fluoroacetophenone and 4-fluorobenzaldehyde (Scheme **1**). The structure was confirmed by its IR spectrum where it showed a characteristic peak for a conjugated carbonyl group at 1662 cm^{-1}. The ^1H NMR displayed two doublets at δ 7.73 ppm and δ 7.90 ppm due to H_α and H_β protons. The coupling constant (*J*) of H_α and H_β is 15.6 Hz which is characteristic to (*E*)-isomer of the chalcone.



Scheme 1: Synthesis of 4,4'-difluoro chalcone.

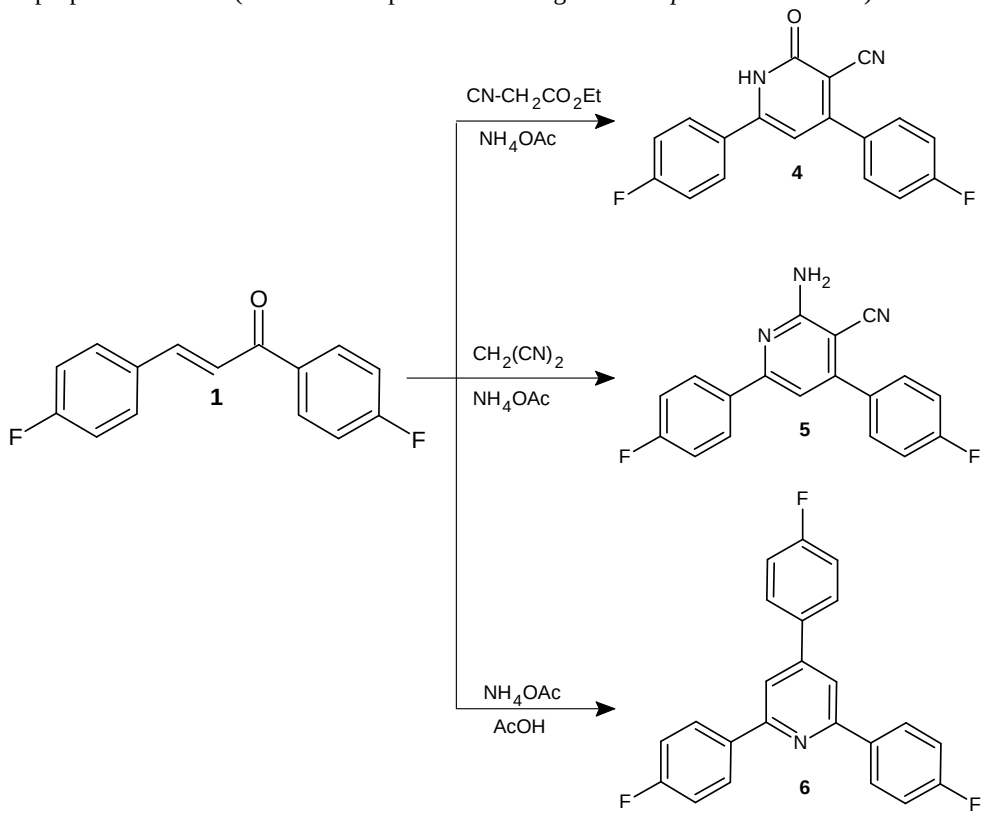
One of the most convenient method for the synthesis of pyrazolines is the reaction of *α,β*-unsaturated ketones with hydrazine hydrate and its derivatives (Scheme **2**) [12, 13]. The formation of **2a,b** and **3a-c** can be rationalized on the basis of two reaction pathways. The first route involves the initial formation of a hydrazone followed by a subsequent *5- endo trig.* ring cyclization, which according to Baldwin's rules is an unfavourable reaction. The second reaction pathway involves a Michael addition of hydrazine on chalcone **1,** followed by a *5-exo-trig.* ring cyclization and dehydration. This is an allowed process according to Baldwin's rules [14]. However, due to the symmetry of the chalcone, the products obtained by either of the mechanisms are the same in all the cases.



Scheme 2: Synthesis of pyrazoline derivatives.

Pyrazoline derivatives **2a** and **2b** were synthesized by the reaction of chalcone **1** with substituted phenylhydrazines in glacial acetic acid under reflux condition. Furthermore, hydrazine hydrate reacted with **1** in the presence of different aliphatic acids resulted in the formation of pyrazoline moiety containing *N*-aliphatic chain **3a-c**. The structure confirmation of compounds **2a,b** and **3a-c** was supported by elemental analyses, IR, ^{1}H NMR as well as mass spectra. The IR spectra of compounds **2a,b** and **3a-c** showed a band near 1600 cm^{-1} accounts for the formation of C=N bond. The formation of 2-pyrazoline ring was confirmed by the appearance of ABX system in ^{1}H NMR due to geminal-vicinal coupling between protons H_A and H_B at C-4 and H_X at C-5. H_A which appeared as doublet of doublets around δ 3.0-3.2 ppm is the proton trans to H_X and geminal to H_B (J_{AB} =17- 18 Hz, J_{AX} = 3-6 Hz). H_B is the proton cis and vicinal to H_X and appeared as doublet of doublets around δ 3.7-3.9 ppm. While, H_X appeared as doublet of doublets around δ 5.3-5.9 ppm (J_{BX} =11-12 Hz) [15, 16]. Further, the structures of compounds **3a** and **3b** were confirmed by single crystal XRD and are given in Fig.1(3a, 3b) [**3a**: Triclinic, $P\bar{1}$, a = 6.2141 (9) Å, b = 6.7802 (8) Å, c = 17.9857 (9) Å, V = 670.39 (13) Å^3 , Z = 2; **3b**: Triclinic, $P\bar{1}$, a = 7.1447 (1) Å, b = 17.2332 (3) Å, c = 18.4871 (4) Å, V = 2173.86 (7) Å^3 , Z = 6] [17, 18].

Usually, condensation of chalcone with malononitrile and ethyl cyanoacetate in refluxing ethanol in the presence of ammonium acetate affords the cyanopyridine derivatives (Scheme **3**) [19]. Similarly, treatment of chalcone **1** with ethyl cyanoacetate and malononitrile in absolute ethanol in presence of ammonium acetate afforded products cyanopyridine derivatives **4** and **5** respectively**.** The structures of them were confirmed by recording their spectral data. IR spectra showed intense bands at 3400-3500 cm^{-1} corresponding to N-H stretching for both the compounds. The ^{1}H NMR spectra substantiated the results of the IR analysis and exhibited a singlet at δ 12.82 ppm for NH proton in compound **4** and a singlet at δ 7.01 ppm for two protons of NH_2 in compound **5**. The mass spectra also supported the proposed structure (The detailed spectral data are given in *Experimental* section).

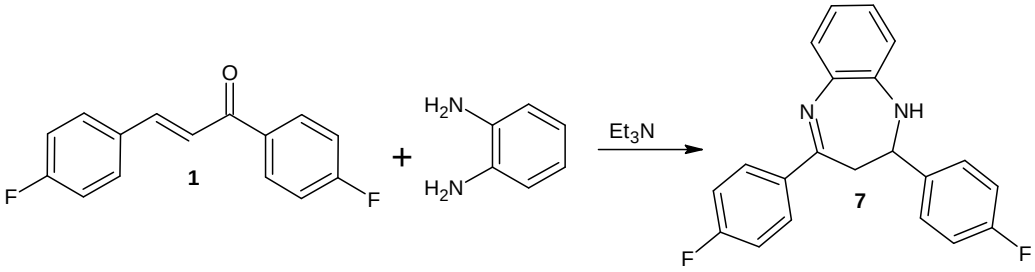


Scheme 3: Synthesis of pyridine derivatives.

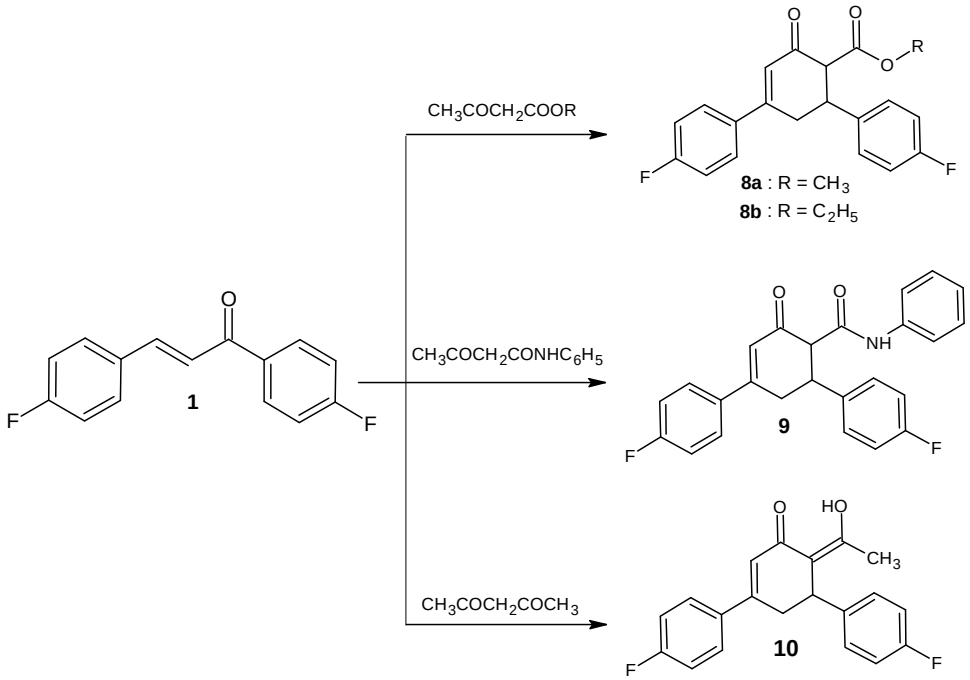
Symmetrical 2,4,6-triarylpyridines were synthesized by heating a mixture of chalcones and ammonium acetate in the presence of a catalytic amount of acetic acid at 100°C for 4 h under solvent-free conditions [20]. Similarly, chalcone **1** treated with ammonium acetate in glacial acetic acid afforded the 2,4,6-triarylpyridine **6** (Scheme **3**)**.** The same

product was also obtained by one-pot condensation of 4,4′-difluoro chalcone, 4-fluoro acetophenone and ammonium acetate in glacial acetic acid which was reported earlier from our group [21]**.**

The reaction of binucleophiles like ortho phenylenediamine with α,β-unsaturated carbonyl compounds can afford 2,3-dihydro-1*H*-1,5-benzodiazepine derivatives in the absence of light [22]. Same methodology was applied to obtain 1,5-benzodiazepine derivative **7** (Scheme **4**). The identity of this compound was confirmed by means of spectral analysis. Formation of **7** was indicated by the presence of an absorption band at 3350 cm^{-1} for N-H stretching in IR spectrum as well as singlet for NH proton at δ 5.88 ppm in ^{1}H NMR spectrum. Further, the structure of compound **7** was confirmed by single crystal XRD and are given in Fig.1(7) [Monoclinic, $P2_1/n$, a = 12.9151 (4) Å, b = 6.0438 (3) Å, c = 21.2851 (7) Å, V = 1660.27 (11) $Å^3$, Z = 4] [23].



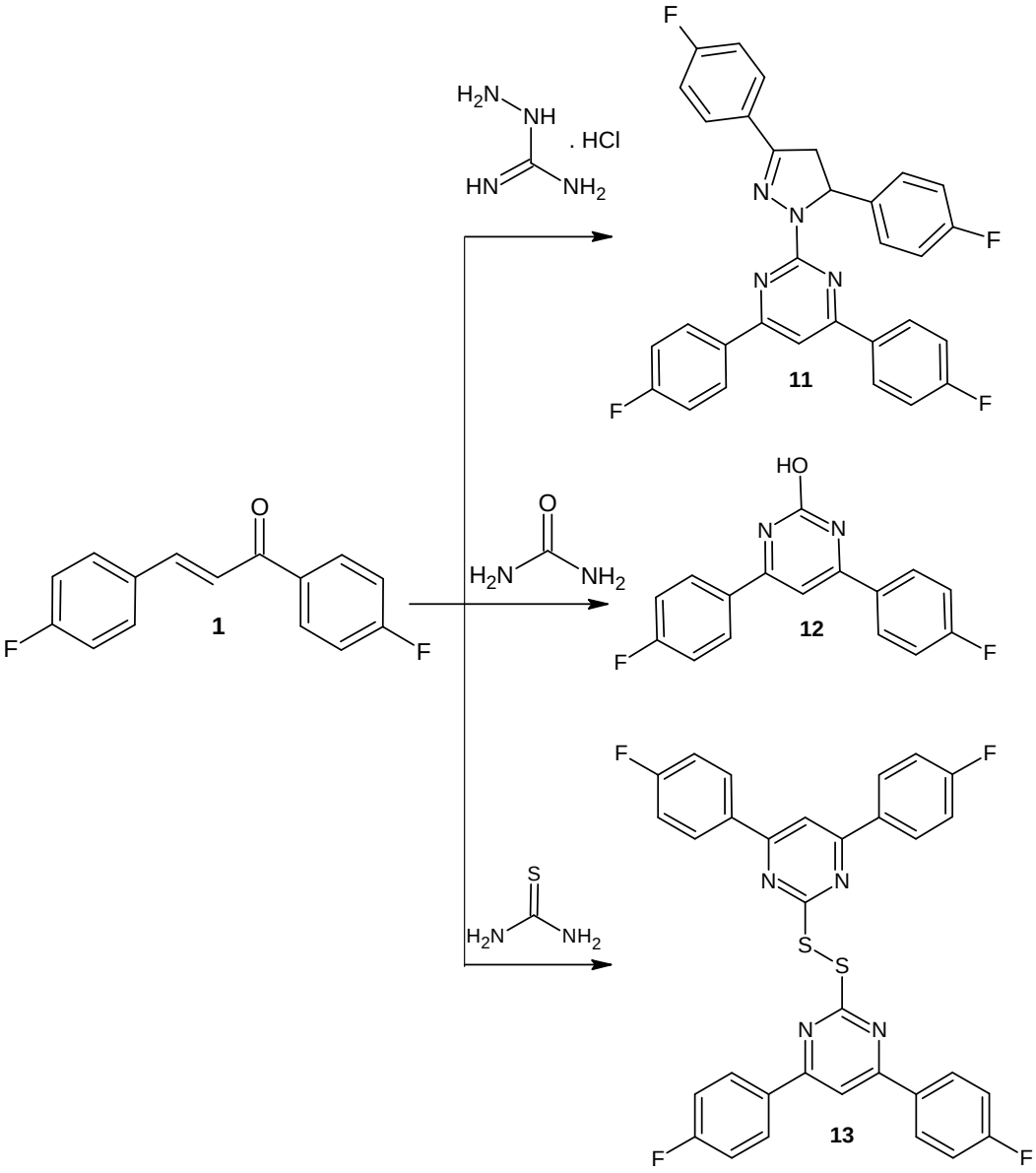
Scheme 4: Synthesis of 1,5-benzodiazepine derivative.



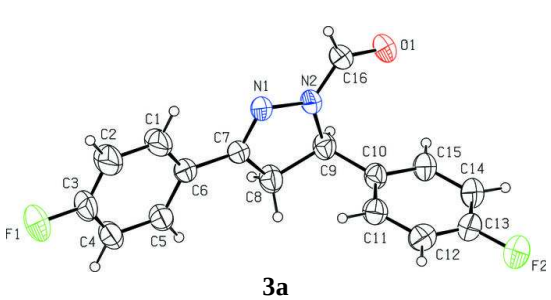
Scheme 5: Synthesis of cyclohexenone derivatives.

The reaction of chalcones with acetoacetic esters is known to lead to three structurally diverse types of compounds, depending on the experimental conditions employed. The catalyst plays a major role in directing the reaction to different end products. While chalcone **1** treated with acetoacetic esters in presence of basic catalyst, the intermediate Michael addition product formed which in turn converted into cyclohexenones **8a, b** through the intramolecular cyclocondensation of the methyl group originating from acetoacetic acid esters and the ketone function of the initial chalcone [24, 25]. Similarly aceto acetanilide and acetyl acetone reacted with chalcone **1** to afford cyclohexenone derivatives **9** and **10** respectively (Scheme **5**). Structural analysis of the newly synthesized cyclohexenones **8a,b, 9** and **10** established by IR, NMR and mass spectral investigations. Further, the structures of compounds **8a, 8b** and **10** were confirmed by single crystal XRD and are given in Fig.1(8a, 8b, 10) [**8a**: Orthorhombic, $Pca2_1$, a = 17.3774 (5) Å, b = 9.0629 (3) Å, c = 22.2238 (7) Å, V = 3500.02 (19) $Å^3$, Z = 8; **8b**:

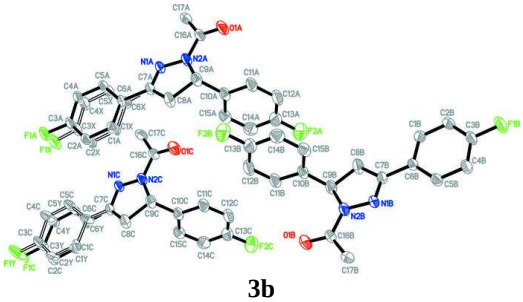
Monoclinic; $P2_1/n$, a = 11.062 (2) Å, b = 11.675 (3) Å, c = 13.854 (3) Å, V = 1787.0 (7) Å^3, Z = 4; **10**: Monoclinic; $P2_1/c$, a = 17.663 (2) Å, b = 6.2371 (6)Å, c = 15.2357 (16)Å, V = 1598.9 (3) Å^3, Z = 4] [26-28].



Scheme 6: Synthesis of pyrimidine derivatives.



3a



3b

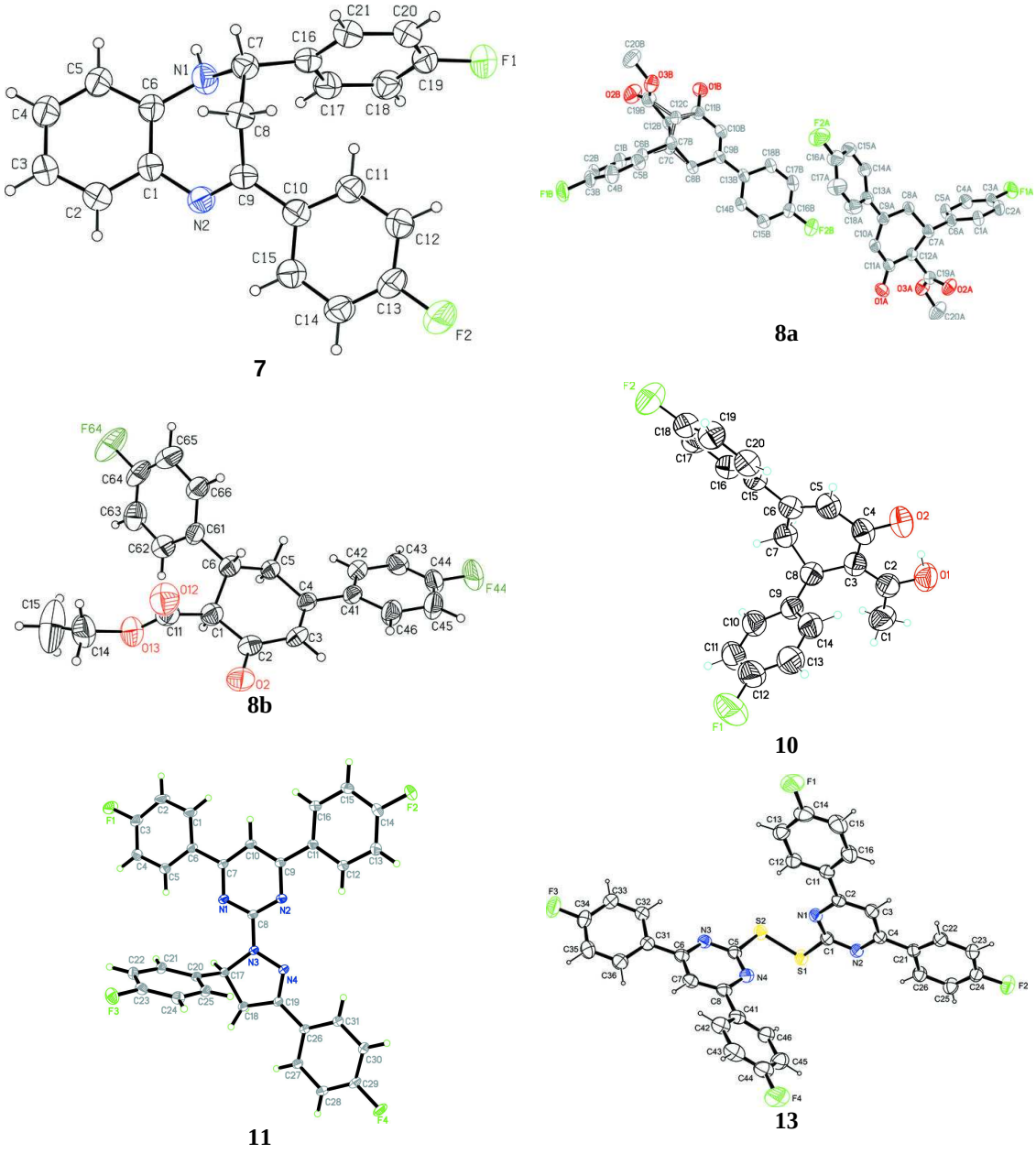


Figure 1: The molecular structure and numbering scheme for the compounds 3a, 3b, 7, 8a, 8b, 10, 11 and 13 with displacement ellipsoids drawn at the 30% probability level for 2a, 2b, 7 & 8a and 50% probability level for 8b, 10, 11& 13.

Chalcone **1** was condensed with amino guanidine hydrochloride under basic condition to obtain pyrazoline derivative. But in this case, the pyrazoline containing -C(NH_2)=NH group further reacted with one more molecule of the chalcone to form pyrazolyl-pyrimidine derivative **11** (Scheme **6**)**.** The structure was confirmed by spectral as well as single crystal XRD data. The absence of carbonyl stretching frequency in the IR spectrum of **11** revealed the cyclization of the chalcone **1**. The ^{1}H NMR spectrum showed a doublet of doublets at δ 3.93 ppm integrating two protons due to CH_2 protons. Even though a chiral centre present in the nearest carbon, the expected two doublets of doublets might have merged in this case. One more doublet of doublet observed at δ 5.91 ppm due to CH protons of pyrazoline ring as the neighbouring two protons were non-equivalent. The mass spectrum showed a molecular ion peak at 524 (M^+) confirming the proposed structure. The structure was further confirmed by single crystal XRD data

(Fig.1(11)) [**11**: Triclinic, $P\bar{1}$, a = 10.1020 (1) Å, b = 10.1106 (1) Å, c = 12.3886 (1) Å, V = 1169.94 (2) $Å^3$, Z = 2] [29].

Similarly, chalcone **1** treated with urea and thiourea under basic condition to obtain pyrimidine derivatives. But, during the condensation of chalcone **1** with thiourea, instead of thiopyrimidine its dimerised product **13** was obtained. The structure of pyrimidine derivatives **12** and **13** were established on the basis of their spectral data. The ^{1}H NMR spectrum of compound **13** showed a singlet at δ 11.89 ppm which was readily assigned to the proton of hydroxyl group. But in the ^{1}H NMR spectrum of compound **13,** signals appeared could be accounted for only 18 aromatic protons, which indicated the dimerised thiopyrimidine derivative. Their IR and mass spectra were conclusive in assigning the structures. The structure was further confirmed by single crystal XRD data (Fig.1 (13)) [**13**: Triclinic, $P\bar{1}$, a = 9.3371 (2) Å, b = 11.3093 (3) Å, c = 13.1984 (3) Å, V = 1354.64 (6) $Å^3$, Z = 2] [30].

3.2. Biological evaluation

3.2.1. Antimicrobial studies

All synthesized compounds were screened for antibacterial activity against Gram-positive *(Bacillus subtilis, Streptococcus haemolytius)* and Gram-negative bacteria *(Pseudomonas aeruginosa, Klebsiella pneumoniae*). The compounds were also tested against two fungal strains *Aspergillus niger* and *Candida albicans* using agar well diffusion method [31, 32]. Further, their MIC values were determined against these organisms by micro dilution method [33] using DMSO as a solvent. Ciprofloxacin and Fluconazole were used as standard antibiotics for antibacterial and antifungal respectively.

Almost all the tested compounds were emerged as active against all tested microorganisms. Even though different functionality present in the molecules, the high antimicrobial activity might be due to the presence of fluorine atom in those molecules. However, this is a very promising preliminary study and further evaluation is needed to use them for clinical use.

3.2.2. DPPH Radical Scavenging Assay

A rapid, simple and inexpensive method to measure antioxidant capacity of substances involves the use of the free radical, 2, 2-diphenyl-1-picrylhydrazyl (DPPH). DPPH is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors. Antioxidants tested on DPPH were also found extremely effective in cell systems. This simple test further provides information on the ability of a compound to donate electrons during antioxidant action [34]. The radical scavenging mechanism is based on the transfer of acidic H-atom from the compound to DPPH radical to form DPPH-H.

Among the tested compounds, compound **12** showed good radical scavenging capacity while compounds **2b, 7** and **13** exhibited moderate radical scavenging capacity with concentration of 50µg/mL in comparison with the standard ascorbic acid (50µg/mL). Other compounds showed low activity. The good radical scavenging capacity of compound **12** is due to the presence of acidic proton of hydroxyl group attached to the pyrimidine ring. The variation exhibited in DPPH scavenging capacity could be attributed to the effect of different moiety present in the compounds.

CONCLUSION

Some functionalized derivatives of 4,4'-difluoro chalcone were prepared by condensing it with hydrazine derivatives, ortho phenylenediamine, ammonium acetate/acetic acid, ethyl cyanoacetate, malononitrile, acetylacetone, esters of acetoacetic acid, acetoacetanilde, aminoguanidine hydrochloride, urea and thiourea. All these derivatives were characterized by spectral and single crystal XRD data. All the synthesized products were screened for their *in vitro* antimicrobial and antioxidant properties. Majority of the tested compounds exhibited significant antimicrobial activity and some of them showed good DPPH scavenging activity. Hence this study has widened the scope of developing easy method to synthesize fluorinated functionalized derivatives as promising antimicrobial and antioxidant agents.

Acknowledgements

BN thanks the UGC for financial assistance through SAP and BSR one time grant for the purchase of chemicals. SS thanks Mangalore University for the research facilities.

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