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SHORT COMMUNICATION

Studies on synthetic and structural characterization of new fluorine substituted phthalides of pharmaceutical interest

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Abstract

An efficient and economical synthesis of some new fluorine substituted phthalides was accomplished from two γ-keto acids, 2-(4-fluorobenzoyl)benzoic acid and 2-(3,5-dinitro-4-flurobenzoyl)benzoic acid. Each acid was reacted with various phenolic compounds in presence of catalytic quantity of concentrated sulphuric acid to get the phthalides. The structures of the synthesized compounds were established on the basis of their elemental analysis, spectral data and chemical reactions. Some of the synthesized phthalides exhibited antibacterial and antifungal activity on antimicrobial screening against human pathogenic bacteria and fungi.

Keywords: Fluorine substituted phthalides, phenols, 2-(4- fluorobenzoyl)benzoic acid, 2-(3,5-dinitro-4-flurobenzoyl)benzoic acid, lactol, sulphuric acid, antibacterial activity, antifungal activity

Introduction

Fluorinated organic compounds are the subject of topical interest due to their unique physical and biological properties[1,2]. It has been observed that presence of fluorine substituents in a lead molecule is capable of changing all the physical properties and influencing various drug-action related phenomenon such as adsorption, distribution, metabolism, and excretion[3]. Consequently, the use of fluorine substituent in drug synthesis has increased considerably during the recent years. Aryl fluoride (Ar-F) moieties are synthetically useful functional groups broadly applicable in medicinal chemistry. They are isosteric to parent hydrocarbons and show improved lipophilicity as well as inertness to metabolic transformations[4–6]. Large number of fluorinated organic compounds exhibit antimicrobial[7,8], antitumour and anti lung cancer[9] activities. Some fluorinated chalcone derivatives are reported to possess anti-inflammatory activity due to their influence on nitric oxide production[10]. Fluorinated 3-aminoindazoles have been identified as lead compounds for the treatment of psychiatric disorders such as obsessive compulsive disorder and attention deficit disorder[11]. In addition, they are reported to be useful in the treatment of diseases associated with protein kinase, e.g., diabetes, cancer and Alzheimer's diseases[12]. The paramount importance of fluorine substituted drugs in human health is evident by the fact that antidepressant fluoxetine (Prozac), the cholesterol–lowering drug atorvastatin (Lipitor), and the antibacterial ciprofloxacin (Ciprobay) are among the most widely used and top-selling fluorine containing drugs[3].

In the present synthetic programme in addition to incorporation of fluorophenyl group, attachment of hydroxyphenyl moiety with phthalide structure has been envisaged because phenols are also endowed with excellent biological properties. It is well recognized that antioxidant activity of several plants is due to their phenolic contents[13]. The use of phenol and *m*-cresol as antiseptics and disinfectants is quite old, and mechanism of their antimicrobial action is known[14]. Curcuphenol is a sesquiterpene phenol recently isolated from sponges and plant sources showed anticancer property[15]. 1-(2-Ethyl-6-heptyl)phenol (EHP), a biologically active compound isolated from cumin (*Cuminum cyminum*) seeds has been found to exhibit anticancer and antibacterial activities[16]. *o*-Dihydroxyphenols are considered the most

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active antioxidants and antimicrobial agents[17]. Catechol and pyrogallol are allelo chemicals which belong to phenolic compounds synthesized in plants[18]. Both of these two compounds are reported to be associated with antibacterial[19,20] and anticancer[21,22] properties. Resorcinol structure is present in large number of compounds of therapeutic value showing anticancer[23,24] and antifungal activity[25,26]. A novel series of quinols with heteroaromatic or (arylsulphonyl) indole substitution at 4 position has been reported to show *in vitro* antitumour activity[27]. Both synthetic and natural phloroglucinols exhibit a vast array of biological activities, viz., anti-inflammatory, anticancer, antimicrobial, antiallergic, enzyme inhibitory, neuroregenerative and antioxidant, etc.[28]

Although organic compounds with phthalide moieties in their structures are well known as analytical reagents and for their photochromatic properties, during recent years, interest in this area has been regenerated and gained a new momentum due to diverse pharmacological properties associated with these compounds. Phthalide [1-(3H)-isobenzofuranone] structures are present in large member of natural products and biologically active compounds[29,30]. A number of medicinal plants are found to contain various phthalides as their active principles[31]. Chiral 3-substituted phthalides are found to possess therapeutic properties and also used as versatile building blocks for large number of medicinally important compounds[32,33]. There is a continuous interest in developing efficient and convenient methods for the synthesis of phthalides[34–38].

Despite the above mentioned facts, to date, almost negligible[38] attention has been paid towards the synthesis of fluorinated phthalides. To address this deficiency, we now document the synthesis of new fluorinated phthalides. In the present study, we have condensed two fluorine containing γ-keto acids, 2-(4-fluorobenzoyl)benzoic acid and 2-(3,5-dinitro-4-fluorobenzoyl)benzoic acid with various mono, di- and trihydroxyphenols in presence of catalytic quantity of concentrated sulphuric acid to afford the fluorine substituted phthalides of biological interest. The used synthetic procedure is simple, efficient, economical and environmentally benign as it does not involve the use of any solvent. To the best of our knowledge, this is the first report on the synthesis of fluorinated phthalides from fluorinated γ-keto acids.

Materials and methods

Melting points were determined in open capillary tubes and are uncorrected. UV spectra were recorded in methanol on a Perkin-Elmer Lambda 15 UV/Vis spectrophotometer (USA). IR spectra were taken in KBr on a Perkin-Elmer FT-IR Spectrum RX-I spectrometer (USA). The ^{1}H NMR spectra were recorded in DMSO-d_6 solutions on a JEOL ECX- 400 spectrometer (Japan) at 400 MHz. Chemical shift (δ) are reported as downfield displacement from TMS that is used as internal standard. Mass spectra were recorded on a SURVEYOR-MSQ (LC-MS) mass spectrometer. The chemicals and solvents were procured from E. Merck (Germany) and Qualigens (India) and used as received without further purification. TLC was used to access the progress of the reactions and to ascertain the purity of the synthesized products. TLC plates coated with alumina-silica gel G (1:1) layers were run in petroleum ether (bp 40–70°C) – acetone (60:40; v: v).

Preparation of 2-(4-fluorobenzoyl)benzoic acid (1a)

A vigorously stirred mixture of fluorobenzene (100 mL) and finely powdered phthalic anhydride (25.2 g, 0.17 mol) was heated to 40–50°C. To this, anhydrous aluminium chloride (52.3 g, 0.34 mol) was added in small parts with continued stirring. The dark brown reaction mixture was heated on a steam bath for 5 h and allowed to stand at room temperature for overnight. Thereafter, it was hydrolyzed with ice and concentrated hydrochloric acid, and steam distilled to remove excess of fluorobenzene that was used as solvent. The crude product after filtration was dissolved in sodium carbonate solution, charcolised and finally acidified with concentrated hydrochloric acid. The precipitated acid **1a** was further purified by crystallization from a mixture of benzene and petroleum ether as colourless needles. Yield, 33.7 g (97%); mp, 138–139°C (lit,[39] 137–137.5°C).

Preparation of 2-(3,5-dinitro-4-fluorobenzoyl)benzoic acid (1b)

2-(4-Fluorobenzoyl)benzoic acid (**1a**) (15.87 g, 0.065 mol) was dissolved in concentrated sulphuric acid (d, 1.83, 36 mL). To this, a mixture of concentrated nitric acid (d, 1.41, 9 mL) and sulphuric acid (d, 1.83, 11 mL) was added gradually with stirring and maintaining the temperature at 40–50°C. The reaction mixture was allowed to stand at 40–50°C for 1 h. After cooling at room temperature, it was poured into ice-cold water with vigorous stirring. The deposited crude acid **1b** was filtered, washed with water and purified by crystallization from ethanol as a light yellow crystalline solid. Yield 12.5 g (57.5%); mp = 159–160°C; IR (KBr) ν_{max}/ cm^{-1}: 3400, 2850, 2750, 1780, 1711, 1680, 1615, 1590, 1530, 1348, 842; ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 7.69–8.6 (m, 6H), 8.69(s, 1H); Anal. Calcd. for $C_{14}H_7FN_2O_7$: C, 50.31; H, 2.11; N, 8.38; Found: C, 50.55; H, 2.20; N, 8.56%.

Synthesis of the fluorinated phthalides (4a-9a and 4b-9b)

Typical synthesis of 3-(2,4-dihydroxyphenyl)-3-(4-fluorophenyl)phthalide (5a)

An intimate and well dried mixture of 2-(4-fluorobenzoyl)benzoic acid **1a** (4.8 g, 0.02 mol) and resorcinol (2.75 g, 0.025 mol) was heated to 110°C in an oil bath to obtain a homogeneous solution. Thereafter, concentrated sulphuric acid (5 drops) was added cautiously and the heating was continued between 110°C and 120°C for 1 h to get a hard and brittle mass on cooling. It was washed thoroughly with water to remove the excess of resorcinol, extracted with 2% aq. sodium

hydroxide and the extract was filtered. The filtrate was cooled and acidified with dilute hydrochloric acid to precipitate the phthalide, **5a**. It was filtered, washed well with water and finally crystallized with aq. ethanol as a pale yellow microcrystalline solid. Yield 5.3 g (78.9%); mp = 145–146°C; UV λ_{max} (MeOH)/ nm: 206, 281; IR (KBr) ν_{max}/ cm^{-1}: 3367, 1740, 983, 756, 694; ^{1}H NMR (400 MHz, DMSO-*d6*): δ 6.13–7.83(m, 11H), 9.47 (s, 2H); MS: ESI-MS, *m/z* (%):337 $[M+H]^+$ (100), 359 $[M+Na]^+$ (24), 391 $[M+Na+MeOH]^+$ (30); Anal. Calcd. for $C_{20}H_{13}FO_4$: C, 71.43; H, 3.90; Found, C, 71.58; H, 3.82 %.

The other phthalides were synthesized by condensing appropriate γ-keto acid (**1a** and **1b**) with phenols (phenol, resorcinol, catechol, quinol, phloroglucinol and pyragallol) following the procedure described above. In case of the condensation of γ-keto acids with phenol, the unreacted phenol after condensation was removed by steam distillation. The relevant data of the synthesized phthalides are given below.

3-(2,4-Dihydroxyphenyl)-3-(3,5-dinitro-4-fluorophenyl) phthalide (5b)

A mixture of the acid 2-(3,5-dinitro-4-fluorobenzoyl) benzoic acid (**1b**) (3.34 g, 0.01 mol) and resorcinol (1.65 g, 0.015 mol) was heated at 120–130°C for 0.5 h in presence of concentrated sulphuric acid (5 drops) to get phthalide **5b** as green solid. Yield 2.75 g (64.6%); mp = 210–212°C; UV: λ_{max} (MeOH)/ nm 207, 252, 382; IR (KBr): ν_{max}/ cm^{-1} 3368, 1763, 1256, 983, 750,685; ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.97–8.60 (m, 9H), 9.80 (s, 2H). MS: ESI-MS, *m/z* (%): 427 $[M+H]^+$ (100), 465 $[M+K]^+$ (5); Anal. Calcd. for $C_{20}H_{11}FN_2O_8$: C, 56.35; H, 2.60; N, 6.57; Found, C, 56.50; H, 2.71; N, 6.63%.

3-(4-Hydroxyphenyl)-3-(4-fluorophenyl)phthalide (4a)

The acid **1a** (2.44 g, 0.01 mol) and phenol (1.41 g, 0.015 mol) were mixed with each other and heated at 80–90°C for 5 h using concentrated sulphuric acid (4 drops) as condensing agent to get **4a** as pinkish white solid. Yield 2.21 g (69.2%); mp = 110–112°C; UV: λ_{max} (MeOH)/ nm 210, 250, 300; IR (KBr): ν_{max}/ cm^{-1} 3351, 1754, 1300, 980, 755, 693; ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.77–7.89 (m, 12H), 9.75 (s, 1H); Anal. Calcd. for $C_{20}H_{13}FO_3$: C, 74.99; H, 4.09; Found, C, 74.77; H, 4.19%.

3-(4-Hydroxyphenyl)-3-(3,5-dinitro-4-fluorophenyl) phthalide (4b)

The acid **1b** (2.17 g, 0.0065 mol) and phenol (0.66 g, 0.007 mol) were mixed with each other and subjected to condensation at 70–80°C for 3 h in presence of concentrated sulphuric acid (3 drops) to afford a yellow solid. Yield 1.60 g (60.0%); mp = 122–124°C; UV: λ_{max} (MeOH)/ nm 210, 281, 305; IR (KBr): ν_{max}/ cm^{-1} 3400, 1710, 1320, 981, 760, 691; ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.75–8.60 (m, 10H), 8.66 (s, 1H); Anal. Calcd. for $C_{20}H_{11}FN_2O_7$: C, 58.54; H, 2.70; N, 6.83; Found, C, 58.64; H, 2.78; N, 6.69%.

3-(3,4-Dihydroxyphenyl)-3-(4-fluorophenyl)phthalide (6a)

A mixture of the acid **1a** (2.44 g, 0.01 mol) and catechol (1.65 g, 0.015 mol) was condensed in presence of concentrated sulphuric acid (3 drops) at 85–95°C for 2 h to afford a blackish grey solid. Yield 2.96 g (88.1%); mp = 110–112°C; UV: λ_{max} (MeOH)/ nm 208, 260, 300; IR (KBr): ν_{max}/ cm^{-1} 3368, 1744, 1335, 994, 753, 694; ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.42–7.89 (m, 11H), 9.13 (s, 2H). Anal. Calcd. for $C_{20}H_{13}FO_4$: C, 71.43; H, 3.90; Found, C, 71.60; H, 3.82%.

3-(3,4-Dihydroxyphenyl)-3-(3,5-dinitro-4-fluorophenyl) phthalide (6b)

The acid **1b** (2.17 g, 0.0065 mol) was mixed with catechol (0.77 g, 0.007 mol) and the resulting mixture was heated at 90–100°C for 40 minutes using concentrated sulphuric acid (3 drops) as condensing agent to obtain phthalide **6b** as pinkish brown solid. Yield 1.44 g (52.0%); mp = 205–207°C; UV: λ_{max} (MeOH)/ nm 210, 280, 350; IR (KBr): ν_{max}/ cm^{-1} 3403, 1773, 1250, 1000, 750, 690; ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.46–8.63 (m, 9H), 9.19 (s, 2H); Anal. Calcd. for $C_{20}H_{11}FN_2O_8$: C, 56.35; H, 2.60; N, 6.57; Found: C, 56.19; H, 2.57; N, 6.61%.

3-(2,5-Dihydroxyphenyl)-3-(4-fluorophenyl)phthalide (7a)

The acid **1a** (2.44 g, 0.01 mol) was condensed with quinol (1.65 g, 0.015 mol) in presence of concentrated sulphuric acid (5 drops) at 135–145°C for 2 h. The usual workup of the reaction mixture gave **7a** as a dark brown solid. Yield 2.46 g (73.3%); mp = 140–142°C; UV: λ_{max} (MeOH)/ nm 205, 260, 300; IR (KBr): ν_{max}/ cm^{-1} 3368, 1744, 1291, 995, 753, 693; ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.58–7.98 (m, 11H), 8.82 (s, 1H), 8.97 (s, 1H); Anal. Calcd. for $C_{20}H_{13}FO_4$: C, 71.43; H, 3.90; Found: C, 71.26; H, 3.83%.

3-(2,5-Dihydroxyphenyl)-3-(3,5-dinitro-4-fluorophenyl) phthalide (7b)

The acid **1b** (2.17 g, 0.0065 mol) was condensed with quinol (0.77 g, 0.007 mol) at 120–130°C in presence of concentrated sulphuric acid (4 drops) for 1 h. The usual workup of the condensed mass gave **7b** as a green solid. Yield, 1.41 g (50.9%); mp = 225–226°C; UV: λ_{max} (MeOH)/ nm 208, 275, 325; IR (KBr): ν_{max}/ cm^{-1} 3385, 1774, 1350, 982, 750, 689; ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.48–8.55 (m, 9H), 8.55 (s, 1H), 8.66 (s, 1H); Anal. Calcd. for $C_{20}H_{11}FN_2O_8$: C, 56.35; H, 2.60; N, 6.57; Found, C, 56.51; H, 2.67; N, 6.78%.

3-(2,4,6-Trihydroxyphenyl)-3-(4-fluorophenyl)phthalide (8a)

An intimate mixture of the acid **1a** (2.44 g, 0.01 mol) and phloroglucinol (1.89 g, 0.015 mol) was heated at 170–180°C for 1 h in presence of concentrated sulphuric acid (3 drops) and the condensed mass was worked up to obtain **8a** as a brick red solid. Yield 2.09 g (59.3%), mp = 245–246°C; UV: λ_{max} (MeOH)/ nm 210, 280, 375; IR (KBr): ν_{max}/ cm^{-1} 3368, 1734, 1289, 1015, 755, 696; ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.59–7.97 (m, 10H), 9.00 (broad and unresolved signal, 3H). Anal. Calcd. for $C_{20}H_{13}FO_5$: C, 68.18; H, 3.72; Found, C, 68.18; H, 3.79%.

3-(2,4,6-Trihydroxyphenyl)-3-(3,5-dinitro-4-fluorophenyl) phthalide (8b)

An intimate mixture of the acid **1b** (2.17 g, 0.0065 mol) and phloroglucinol (0.88 g, 0.007 mol) was heated at 155–165°C for 0.5 h using concentrated sulphuric acid (3 drops) to get **8b** as a brown solid. Yield 2.07 g (72.3%); mp = >300°C; UV: λ_{max} (MeOH)/ nm 210, 281, 380; IR (KBr): ν_{max}/ cm^{-1} 3377, 1717, 1350, 1000, 747, 687; ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.10 (br s, 3H), 6.93–8.64 (m, 8H); Anal. Calcd. for $C_{20}H_{11}FN_2O_9$: C, 54.31; H, 2.51; N, 6.33; Found: C, 54.60; H, 2.58; N, 6.40 %.

3-(2,3,4-Trihydroxyphenyl)-3-(4-fluorophenyl)phthalide (9a)

The acid **1a** (2.44 g, 0.01 mol) was intimately mixed with pyrogallol (1.89 g, 0.015 mol). Heating of this mixture at 115–125°C for 0.5 h in presence of concentrated sulphuric acid (3 drops) and work up in usual manner afforded the phthalide **9a** as a yellow solid. Yield 2.06 g (58.5%); mp = 170–172°C; UV: λ_{max} (MeOH)/ nm 203, 281, 382; IR (KBr): ν_{max}/ cm^{-1} 3422, 1730, 1300, 1000, 752, 695; ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.51–7.82 (m, 10H), 8.41 (s, 3H). Anal. Calcd. for $C_{20}H_{13}FO_5$: C, 68.18; H, 3.72; Found, C, 68.45; H, 3.83%.

3-(2,3,4-Trihydroxyphenyl)-3-(3,5-dinitro-4-fluorophenyl) phthalide (9b)

The acid **1b** (2.17 g, 0.0065 mol) was intimately mixed with pyrogallol (0.88 g, 0.007 mol). On heating the mixture at 110–120°C in presence of concentrated sulphuric acid (3 drops) the phthalide **9b** was instantly obtained within 10 minutes as a brown solid. Yield 1.77 g (64.2%). MP: >300°C; UV: λ_{max} (MeOH)/ nm 203, 280, 382; IR (KBr): ν_{max}/ cm^{-1} 3365, 1718, 1261, 990, 759, 690; ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.92 (br s, 3H), 7.00–8.10 (unresolved m, 8H); Anal. Calcd. for $C_{20}H_{11}FN_2O_9$: C, 54.31; H, 2.51; N, 6.33 Found: C, 54.12; H, 2.48; N, 6.45%.

Acetylation of phthalide 5a and 5b

Synthesis of 3-(2,4-diacetoxyphenyl)-3-(4-fluorophenyl) phthalide (10a)

The phthalide, **5a** (1 g, 0.003 mol) was mixed with acetic anhydride (20 mL) and freshly fused sodium acetate (3.00 g). The resulting mixture was refluxed at 130–140°C for 3.5 h, poured into ice-cold water with stirring and allowed to stand for 2 h. The deposited crude diacetyl compound **10a** was filtered and purified by crystallization from aq. acetone as an off white crystalline solid. Yield 0.82 g (65.5%); mp = 120–122°C; UV: λ_{max} (MeOH)/ nm 204, 266, 287; IR (KBr): ν_{max}/ cm^{-1} 1777, 1288, 1189, 978, 759, 693; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.04 (s, 6H), 6.88–7.92 (m, 11H); Anal. Calcd. for $C_{24}H_{17}FO_6$: C, 68.57; H, 4.08; Found, C, 68.70; H, 3.98%.

Synthesis of 3-(2,4-diacetoxyphenyl)-3-(3,5-dinitro-4-fluorophenyl)phthalide (10b)

Following the procedure given above, the phthalide **5b** (1.02 g, 0.0024 mol) was acetylated with acetic anhydride (25 mL) in presence of fused sodium acetate (3 g). The resulting mixture was refluxed at 130–140°C to afford the diacetyl compound **10b**. It was recrystallised from aq. acetone as a light brown solid. Yield 0.91 g (74.1%); mp = 165–167°C; UV: λ_{max} (MeOH)/ nm 203, 260, 285; IR (KBr): ν_{max}/ cm^{-1} 1780, 1290, 1190, 978, 760, 693; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.05 (s, 6H), 7.08–8.64 (m, 9H); Anal. Calcd. for $C_{24}H_{15}FN_2O_{10}$: C, 56.48; H, 2.96; N, 5.49; Found, C, 56.05; H, 2.72; N, 5.92%.

Bromination of phthalide 5a and 5b

Synthesis of 3-(3,5-dibromo-2,4-dihydroxyphenyl)-3-(4-fluorophenyl)phthalide (11a)

The phthalide **5a** (1 g, 0.003 mol) was finely powdered and dissolved in ethanol (10 mL), and bromine (2 mL) was added drop by drop with shaking. The resultant mixture was allowed to stand overnight at room temperature. The dibromo compound **11a** separated as viscous oil. It was repeatedly washed with cold water and dissolved in aq. sodium hydroxide and filtered. The filtrate was acidified with dilute hydrochloric acid to get the dibromo compound as a brown powder which was finally crystallized from ethanol. Yield 0.91 g (61.4%); mp = 135–137°C; UV: λ_{max} (MeOH)/ nm 204, 246, 285; IR (KBr): ν_{max}/ cm^{-1} 3400, 1725, 1282, 1020, 758, 713; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.28–7.92 (m, 9H), 9.94 (s, 2H); Anal. Calcd. for $C_{20}H_{11}FO_4Br_2$: C, 48.62; H, 2.24; Br, 32.34; Found: C, 48.83; H, 2.28; Br, 32.50%.

Synthesis of 3-(3,5-dibromo-2,4-dihydroxyphenyl)-3-(3,5-dinitro-4-fluorophenyl)phthalide (11b)

The phthalide **5b** (1.02 g, 0.0024 mol) was dissolved in ethanol (20 mL) and treated with bromine (2 mL) to get the dibromo compound **11b** following the procedure used for the synthesis of **11a** as described above. It was a brown solid. Yield 0.99 g (71.2%); mp = 155–157°C; UV: λ_{max} (MeOH)/ nm 211, 255, 300; IR (KBr): ν_{max}/ cm^{-1} 3448, 1774, 1350, 980, 750, 690; ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.70–8.65 (m, 7H), 9.68 (s, 2H). Anal. Calcd. for $C_{20}H_9FN_2O_8Br_2$: C, 41.13; H, 1.55; N, 4.80; Br, 27.36; Found, C, 41.30; H, 1.60; N, 4.75; Br, 27.40%.

Alkaline degradation of 5a and 5b

A mixture of phthalide **5a** (1 g), KOH pellets (10 g) and water (10 mL) was strongly heated at 250°C for 3 h. The contents were cooled, dissolved in water and filtered. The filtrate was acidified with dilute hydrochloric acid when a solid compound (I) was obtained. It was filtered and the filtrate so obtained was extracted with ether (3 × 25 mL). The combined organic layer was dried over anhydrous sodium sulphate and distilled off to afford another solid compound (II). The compounds I and II were identified as 2-(4-hydroxybenzoyl)benzoic acid (**12a**) and resorcinol, respectively by direct comparison (mp, mixed mp, co-TLC and co-IR spectra) with their authentic samples. In a similar manner the phthalide **5b** (1 g) was mixed with KOH pellets (10 g) and water (10 mL) to make a thick paste, and strongly heated at 250°C for 3 h. In this case also, the workup of the

residue gave two compounds which were identified as 2-(3,5-dinitro-4-hydroxybenzoyl)benzoic acid (**12b**) and resorcinol. The authentic samples of **12a** and **12b** were prepared by the literature procedure[39] from **1a** and **1b**, respectively.

Procedure for biological activity

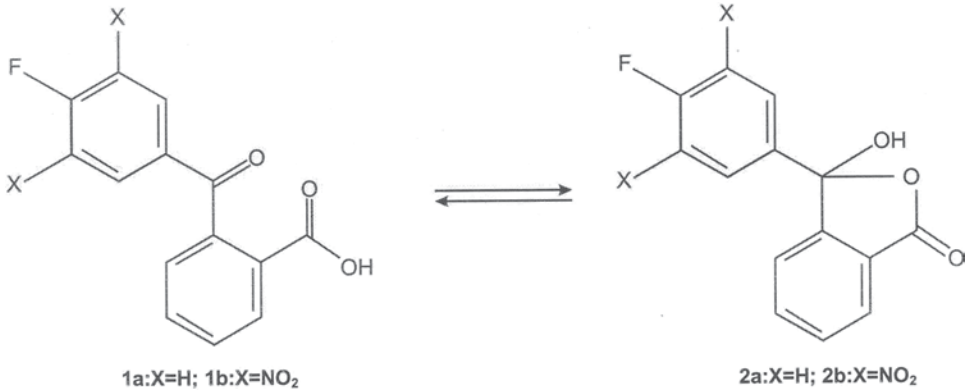
The fluorinated phthalides **6a**, **6b**, **9a** and **9b** were evaluated for their *in vitro* antibacterial activity against *Staphylococcus aureus* ATCC 29213 (Sa), methicillin-resistant *S. aureus* ATCC 33591 (MRS), *Escherichia coli* ATCC 35218 (Ec), *Pseudomonas aeruginosa* ATCC 27853 (Pa), and *Mycobacterium intracellulare.* The antifungal activity was tested against *Candida albicans* ATCC 90028 (Ca), *C. glabrata* ATCC 90030 (Cg), *C. krusei* ATCC 6258 (Ck), *Cryptococcus neoformans* ATCC 90113 (Cn), and *Aspergillus fumigatus* ATCC 204305 (Af).

All organisms were tested using modified versions of the CLSI (formerly NCCLS) methods. For all organisms excluding *M. intracellulare* and *A. fumigatus*, optical density was used to monitor the growth[40,41]. Media supplemented with 5% Alamar Blue™ (BioSource International, Camarillo, CA) was utilized for growth detection of *M. intracellulare*[42,43] and *A. fumigatus*[44]. Samples (dissolved in DMSO) were serially-diluted in 20% DMSO/saline and transferred (10 μL) in duplicate to 96-well flat bottom microplates. Inocula were prepared by correcting the OD_{630} of microbe suspensions in incubation broth [RPMI 1640/0.2% dextrose/0.03% glutamine/MOPS at pH 6.0 (Cellgro) for *Candida* spp., Sabouraud Dextrose for *C. neoformans*, cation-adjusted Mueller-Hinton (Difco) at pH 7.3 for *Staphylococcus* spp., *E. coli*, and *P. aeruginosa*, 5% Alamar Blue™(BioSource International, Camarillo, CA) in Middlebrook 7H9 broth with OADC enrichment, pH 7.0 for *M. intracellulare*, and 5% Alamar Blue™/RPMI 1640 broth (0.2% dextrose, 0.03% glutamine, buffered with 0.165 M MOPS at pH 7.0) for *A. fumigatus* to afford an assay volume of 200 μL and final target inocula of: *Candida* spp. and *C. neoformans*: 1.5 × 103, *M. intracellulare*: 2.0 × 106, *Staphylococcus* spp., *E. coli*, *P. aeruginosa*: 5.0 × 105 CFU/mL, and *A. fumigatus*: 2.7 × 104 CFU/mL. Final sample test concentrations are 1/100th the DMSO stock concentration.

Drug controls (Ciprofloxacin (ICN Biomedicals, OH) for bacteria and Amphotericin B (ICN Biomedicals, OH) for fungi) are included in each assay. All organisms are read at either 530 nm using the Biotek Powerwave XS plate reader (Bio-Tek Instruments, Vermont) or 544 ex/590 em, (*M. intracellulare, A. fumigatus*) using the Polarstar Galaxy Plate Reader (BMG LabTechnologies, Germany) prior to and after incubation: *Candida* spp. at 35°C for 46–50 h, *Staphylococcus* spp., *E. coli*, and *P. aeruginosa* at 35°C for 16–20 h, *C. neoformans* at 35°C for 70–74 h, *A. fumigatus* at 35°C for 46–50 h, and *M. intracellulare* at 37°C and 10% CO_2 for 70–74 h. IC_{50} (concentrations that afford 50% inhibition relative to controls) were calculated using XLfit 4.2 software (IDBS, Alameda, CA) using fit model 201. The MIC was defined as the lowest test concentration that allows no detectable growth (for *M. intracellulare* and *A. fumigatus*, no colour change from blue to pink).

Results and discussion

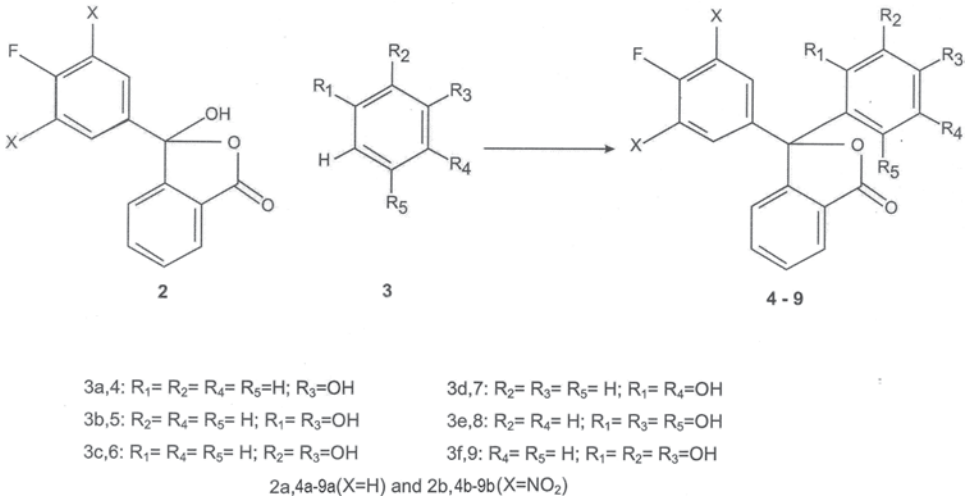
During the course of our present work, it has been found that 2-(4-fluorobenzoyl)benzoic acid (**1a**) and 2-(3,5-dinitro-4-fluorobenzoyl)benzoic acid (**1b**) reacted with phenol, resorcinol, catechol, quinol, phloroglucinol and pyrogallol in presence of a few drops of concentrated sulphuric acid to produce new fluorine substituted phthalides (**4a-9a** and **4b-9b**). In the synthesized phthalides, carbon- 3 of the phthalide structure is attached to two different phenyl rings, one being phenolic and the other non-phenolic having a fluorine atom with or without nitro groups. Thus, the synthesized compounds may be regarded as unsymmetrically substituted phthalides. The phenols (**3**) were taken in slight excess of molecular proportion than the γ-keto acids **1a** and **1b**. The occurrence of keto-lactol tautomerism in γ-keto and γ-formyl acids, and their participation in some chemical reactions through their cyclic lactol form is well documented[45,46]. The open keto-acid (**1**) and the cyclic lactol (**2**) tautomeric forms of the 2-(4-fluorobenzoyl) benzoic acid and 2-(3,5-dinitro-4-benzoyl)benzoic acid maybe depicted as given in Scheme 1. These two acids (**1**) reacted with phenols (**3**) through their cyclic lactol



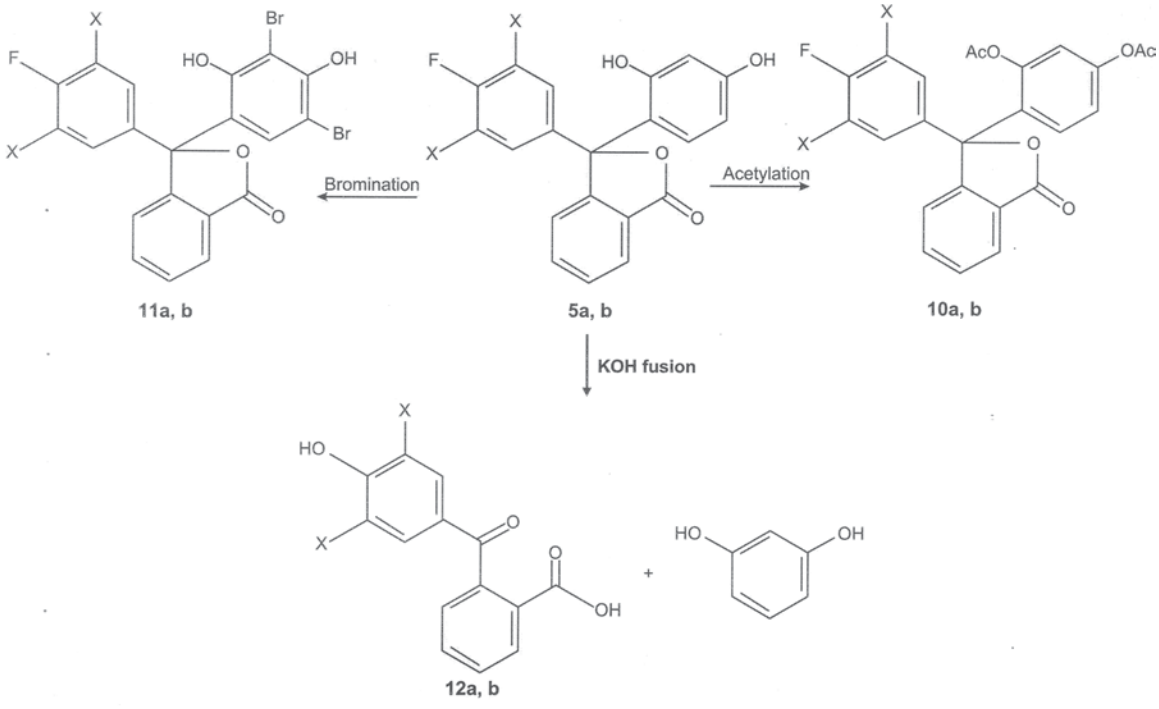
Scheme 1. Keto-acid and lactol tautomeric forms of 2-(4-fluorobenzoyl)benzoic acid and 2-(3,5-dinitro-4-fluorobenzoyl)benzoic acids.

tautomeric form (**2**) to give the fluorinated phthalides (**4a-9a** and **4b-9b**) as shown in Scheme 2. In the light of the reported pharmacological activities associated with constituent moieties present in **4a-9a** and **4b-9b**, these compounds as well as their diacetyl (**10a** and **10b**) and dibromo derivatives (**11a** and **11b**) (Scheme 3) are expected to find use as therapeutic agents.

It has been found that efficient synthesis of the phthalides depends mainly on three factors such as proper condensation temperature, proper condensation time and proper quantity of the catalyst. Therefore, during the present study, these three factors have been worked out for each of the synthesized phthalides. It was also observed that during the reaction of the γ-keto acid **1b** with phenols (**3**) the presence of electron withdrawing nitro groups in **1b** considerably reduced the condensation time during the synthesis of the phthalides (**4b-9b**) in comparison to reaction of **1a** with phenols giving phthalides (**4a-9a**). This can be attributed to the fact that presence of electron withdrawing groups in γ-keto acids augments the formation of cyclic lactol tautomer and thereby increase the rate of those reactions wherein the cyclic lactol form is involved. The structures proposed to the synthesized fluorine substituted phthalides were established on the basis of elemental analysis, UV, IR, ^{1}H NMR and Mass spectral data and chemical reactions, viz., acetylation, bromination and fusion with potassium hydroxide. The representative phthalides **5a** and **5b** on



Scheme 2. Synthesis of fluorinated phthalides 4-9.



Scheme 3. Acetylation, bromination and KOH fusion of fluorinated phthalides 5a, b.

acetylation and bromination gave their corresponding diacetyl derivatives (**10a** and **10b**) and dibromo derivatives (**11a** and **11b**), respectively. The potassium hydroxide fusion of **5a** and **5b** degraded them to γ-keto acids, 2-(4-hydroxybenzoyl)benzoic acid (**12a**) and 2-(3,5-dintro-4-hydroxybenzoyl)benzoic acid (**12b**), respectively and resorcinol. The acetylation, bromination, and potassium hydroxide degradation reactions of **5a** and **5b** are given in Scheme 3.

The starting 2-(4-fluorobenzoyl)benzoic acid (**1a**) was prepared by Friedel-Crafts condensation of fluorobenzene with phthalic anhydride in presence of anhydrous aluminium chloride following a literature method[39] with some modifications in workup procedure in order to improve the yield and purity of the product. The acid **1a** was nitrated by the method of De Tar and Relye[47] to afford 2-(3,5-dinitro-4-fluorobenzoyl)benzoic acid (**1b**). These workers used this method for the synthesis of 2-(3-nitrobenzoyl)benzoic acid from 2-benzoylbenzoic acid. It is interesting to note that during the present study we have obtained a dinitro product (**1b**) instead of the mononitro acid[47].

The IR spectra (in KBr) of the synthesized fluorinated phthalides **4a-9a** and **4b-9b** and **11a** and **11b** showed a broad and strong absorption band in the region 3351–3448 cm^{-1} due to bonded OH stretching vibrations. The diacetyl compounds **10a** and **10b** did not exhibit any IR absorption in hydroxyl region. All the compounds (**4a-11a** and **4b-11b**) displayed a sharp and strong band near 1730–1780 cm^{-1} which is characteristic of lactonic carbonyl group present in phthalide structures. Besides the presence of this band, two bands were noticed at 693–713 cm^{-1} and 750–760 cm^{-1} which can be attributed to *o*-disubstituted phthalide ring. The stretching vibrations of C-O-C of the lactone structure and C-O bond of phenolic group of the phthalides gave absorption peaks near 980–1015 cm^{-1} and 1288–1300 cm^{-1}, respectively. The UV spectra (in methanol) of all the compounds revealed same pattern of absorption at 203–211, 250–281 and 285–382 nm. In the ^1H NMR spectra (400 MHz, DMSO-d_6) of the synthesized phthalides (**4a-11a** and **4b-11b**) the aromatic protons formed a complex multiplet in the region δ 6.42–8.65. Their hydroxyl protons generally appeared as a singlet near δ 8.41–9.80. In **10a** and **10b**, protons of acetoxyl groups formed a singlet at about δ 2.04–2.05. The representative phthalides **5a** and **5b** were subjected to mass spectral analysis to confirm the proposed structures on the basis of fragmentation pattern and molecular weight obtained from molecular ion. The compound **5a** gave a molecular ion at *m/z* 337, while in case of **5b** molecular ion appeared at *m/z* 427. In both the compounds (**5a** and **5b)** molecular ion peak appeared as base peak exhibiting 100% abundance.

Biological activity

The synthesized fluorinated phthalides (**4a-11a** and **4b-11b**) are expected to possess diverse biological activities due to combination of fluorophenyl, hydroxyphenyl and phthalide moieties within a single entity. During the present study, the phthalides, 3-(3,4-dihydroxyphenyl)-3-(4-fluorophenyl)phthalide (**6a**); 3-(3,4-dihydroxyphenyl)-3-(3,5-dinitro-4-fluorophenyl) phthalide (**6b**); 3-(2,3,4-trihydroxyphenyl)-3-(4-fluorophenyl)phthalide (**9a**) and 3-(2,3,4-trihydroxyphenyl)-3--(3,5-dinitro-4-fluorophenyl)phthalide (**9b**) were tested for their *in vitro* antibacterial activity against *S. aureus* ATCC 29213 (Sa), methicillin-resistant *S. aureus* ATCC 33591 (MRS), *E. coli* ATCC 35218 (Ec), *P. aeruginosa* ATCC 27853 (Pa), and *M. intracellulare* ATCC 23068 (Mi) using DMSO as solvent. The *in vitro* antifungal activity of the phthalides **6a**, **6b**, **9a** and **9b** was determined against *C. albicans* ATCC 90028 (Ca), *C. glabrata* ATCC 90030 (Cg), *C. krusei* ATCC 6258 (Ck), *Cryptococcus neoformans* ATCC 90113 (Cn), and *Aspergillus fumigatus* ATCC 204305 (Af). The results of this antimicrobial assay are presented in Tables 1 and 2. The compounds selected for the antimicrobial screening contain catechol and pyrogallol structural units in their structures. The rationale for this choice is the fact that catechol and pyrogallol are allelo chemicals belonging to phenolic compounds synthesized in plants[18], and significant antibacterial activity is associated with them[19,20].

In the present study, it has been noticed that presence of nitro groups in phthalides considerably decrease the antifungal and antibacterial activity. The antifungal activity of the dinitro phthalides is much lower than their antibacterial activity. Dihydroxy substituted phthalides were found to possess higher antibacterial activity in comparison of trihydroxy substituted phthalides.

Mode of action of phthalides on biological systems

Although there are numerous reports on the bioactivities of phthalides, but investigations concerning their mode

Table 1. Antifungal and antibacterial activity of some of the synthesized phthalides (IC_{50} μg/mL).

	IC_{50} in μg/mL									
Compounds	*C. albicans*	*C. glabrata*	*C. krusei*	*A. fumigatus*	*C. neoformans*	*S. aureus*	MRS	*E. coli*	*P. aeruginosa*	*M. intracellulare*
6a	174.43	105.12	94.26	53.80	54.66	8.11	10.69	200	99.75	150.89
6b	200	200	200	200	200	48.01	54.53	200	200	200
9a	161.70	36.54	102.05	200	200	95.77	88.84	200	200	200
9b	200	200	200	200	200	29.73	71.97	200	200	200
Amphotericin B	0.20	0.28	0.56	0.67	0.32	—	—	—	—	—
Ciprofloxacin	—	—	—	—	—	0.11	0.10	0.005	0.11	0.32

Table 2. Antifungal and antibacterial activity of some of the synthesized novel phthalides (MIC μg/mL).

	MIC in μg/mL									
Compounds	*C. albicans*	*C. glabrata*	*C. krusei*	*A. fumigatus*	*C. neoformans*	*S. aureus*	MRS	*E. coli*	*P. aeruginosa*	*M. intracellulare*
6a	200	200	200	200	100	25.00	25.00	200	200	200
6b	200	200	200	200	200	150.00	50.00	100	200	200
9a	200	133.33	200	200	200	166.67	133.33	200	200	200
9b	200	200	200	200	200	200	200	200	200	200
Amphotericin B	0.52	0.63	1.25	1.25	0.63	—	—	—	—	—
Ciprofloxacin	—	—	—	—	—	0.33	0.42	0.013	0.83	0.50

of action are very scanty. However, it has been suggested that presence of five membered lactone ring in phthalides is responsible for their biological activity[48]. Antibacterial activity of phthalides has been explained by proposing a reaction between phthalides and amino acid cysteine, and certain enzymes (containing mercapto group) which are present in bacterial proteins and are necessary for their growth and normal activity. As a consequence of this reaction, the growth process of bacteria is inhibited. In an experiment involving the action of butylidenephthalide on hairless mouse, formation of a cysteine adduct as a urinary metabolite has been detected in the urine of the mouse[49]. Examination of the structures of the new phthalides (**4a-11a** and **4b-11b**) synthesized during the present study reveals that owing to their suitable structural feature, they are also capable of reacting with cysteine to form their corresponding adducts, and thereby exhibiting antimicrobial activity.

The precise biological activity of 3-substituted phthalides is often crucially related with chirality[30,50]. Chiral 3-substituted phthalides such as isochracinic acid[51], herbaranic acid[51], cytosporone E[52] have been found to exhibit antibacterial activity, and fuscinarin[53] is a potent human CCR5 antagonist, effectively blocking HIV entry into host cell. Since the phthalides reported in this paper possess a chiral centre (C-3) in their structure, and therefore, it may be assumed that the antimicrobial activity of these racemic compounds is potentially influenced by it.

Conclusion

The present report constitute the first successful attempt to synthesize new fluorine substituted phthalides from fluorine containing 2-aroylbenzoic acids by a simple and straightforward protocol without producing any byproducts. Besides having the advantages of simplicity, good yields, use of cheap and easily accessible starting chemicals, this method is environmentally benign as it does not involve the use of any solvent. The synthesized fluorinated compounds are anticipated to have diverse applications in medicinal fields. A few of them have been found to exhibit some antibacterial and antifungal activity on *in vitro* antimicrobial screening. Specially, **6a** has shown promising activity against *S. aureus* and MRS. In view of the growing incidences of drug resistance in microorganisms, and remarkable bioenhancing property shown by some less active or even inactive compounds, further appropriate investigations on anticipated biological properties including *in vivo* antimicrobial screening of the synthesized fluorinated phthalides are warranted to explore their therapeutic potential.

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Declaration of interest

The authors report no conflicts of interest.

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