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Synthesis and Larvicidal Activity of Morpholinophenyl and Benzyl-Fluoro Pyrimidine Derivatives

Satya Kumar Avula¹, Shashi Kanth Boddu², Prashanthi Yarasani^{3*}

¹Department of Biological Sciences and Chemistry, College of Arts and Sciences, University of Nizwa Birkat Al-Mouz, Nizwa, Sultanate of Oman

²Department of Chemistry, Annie Besant PG College, Osmania University, Telangana State, India

³Department of Chemistry, Mahatma Gandhi University, Nalgonda, Telangana State, India

ABSTRACT

A series of 5-fluoro-N¹-(3-(4-substitutedbenzylamino)phenyl)-N²-(4-morpholinophenyl) pyrimidine-2,4-diamine derivatives 7a-J were prepared and tested for their larvicidal activity against third instar larvae at 24, 48 and 72 hours and values were compared with Malathion as a positive control. Compounds 7a, 7c, 7f, 7g and 7i exhibited good larvicidal significant activity. Interestingly, compounds 7b, 7d, 7e, and 7h showed excellent activity very likely due to the presence of functional groups attached to the benzyl ring.

Keywords: Larvicidal activity, Morpholinophenyl, Pyrimidines, Pharmacophore, Synthesis

INTRODUCTION

Mosquito-borne diseases, such as dengue fever, malaria, encephalitis, yellow fever are major threats to millions of people throughout the world. The common reasons being the tropical, subtropical climate, poor drainage system especially during rainy seasons, adversely maintained fish ponds, irrigation ditches and rice fields which provide abundant mosquito breeding places. Although chemical vector control programs have been carried out for long time, these mosquito-vector diseases remain because of the refusal by householders to house spraying with synthetic insecticides [1,2]. The main vector of these diseases is the mosquito *Aedes aegypti* (*A. aegypti*) that transmits dengue fever, dengue hemorrhagic fever and yellow fever over many areas of tropics and subtropics [3]. Among them dengue fever is the most dangerous and case list is also increasing day by day. In the last five years most of the insecticides did not show any effect on insects like mosquitoes due to increased drug resistance [4,5]. These studies have emphasized for the new, more effective larvicidal agents. In the pursuit of design of new drugs, the development of hybrid molecules through the combination of different pharmacophores in one frame, could lead to compounds with interesting biological properties. Prompted by these observations, in the present study, the synthesis and larvicidal screening of new derivatives 7a-J bearing different pyrimidines and morpholinophenyl pharmacophores moieties as hybrid molecules possessing larvicidal activity were investigated.

The 4-morpholinophenyl derivatives represent an interesting class of compounds possessing a wide spectrum of biological activities. A large number of morpholinophenyl derivatives exhibited antibacterial [6], antifungal [7], anticonvulsant [8], analgesic [9], anticancer [10] and antiviral activity [11]. Several types of pyrimidines were proved to possess valuable pharmacological properties, such as antiviral [12], antioxidant [13], antimalarial [14], anticonvulsant [15], against Alzheimer's disease [16], antidepressant [17], antimicrobial [18], antitubercular [19], anti-inflammatory [20], antiplatelet [21], antibacterial, antifungal [22], anticancer agents [23], antiproliferative agent [24] and anti-HIV agents [25]. Integration of these moieties may show synergistic effect and hence in the present study an attempt is made for the synthesis of some novel heterocyclic molecules containing the 4-morpholinophenyl and pyrimidine systems, with a hope that they may exhibit a synergistic and larvicidal activity. In the present communication we report the synthesis and larvicidal activity of new fluoro pyrimidines linked with morpholinophenyl and benzyl derivatives.

EXPERIMENTAL

General experimental protocols

Melting points were determined in open capillaries on a Mel-Temp apparatus and are uncorrected. All the reactions were monitored by Thin Layer Chromatography (TLC). The silica gel F₂₅₄ plates were used for TLC in which the spots were examined under UV light and then developed by an iodine vapor. Column chromatography was performed with silica gel (BDH 100-200 mesh). Solvents were purified according to standard procedures. The spectra were recorded with the following instruments; NMR: Varian Gemini 400 MHz (¹H), 75 MHz (¹³C) and 376 MHz (¹⁹F) spectrometer; ESIMS: VG-Autospec micromass, analyses of all the compounds were recorded in LCQ Fleet, Thermo Fisher Instruments Limited. Organic extracts were dried over anhydrous Na₂SO₄.

General procedure for the synthesis of 2-chloro-5-fluoro-N-(3-nitrophenyl)pyrimidin-4-amine (3)

3-Nitrobenzenamine 2 (20 mmol) was added to a mixture of 2,4-dichloro-5-fluoropyrimidine 1 (10 mmol) and K₂CO₃ (15 mmol) in dimethyl sulfoxide (10 mL). The reaction mixture was stirred at 90°C and followed by TLC. After completion of the reaction (monitored by TLC), the mixture was diluted with ethyl acetate and washed with water. The ethyl acetate fraction was separated, dried over Na₂SO₄ and was evaporated. The obtained compound was recrystallized to get 2-chloro-5-fluoro-N-(3-nitrophenyl)pyrimidin-4-amine as a white solid (2.83 g, 88%), m.p. 167-169°C; ¹H NMR (CDCl₃, 400 MHz): δ 8.24 (brs, 1H), 7.62 (d, 1H, J=8.70 Hz), 7.43 (s, 1H), 7.40 (t, 1H, J=8.2 Hz), 7.32-7.28 (m, 1H), 7.20 (d, 1H, J=7.0 Hz); ¹³C NMR (CDCl₃, 75 MHz): δ 156.6, 155.5, 153.4, 147.6, 143.8, 141.8, 130.2, 122.8, 114.8, 111.7; ¹⁹F NMR (CDCl₃, 376 MHz): δ -118.34 (s, 1F); ESIMS: m/z 291 (M+Na)⁺. Anal. Calcd. for C₁₀H₆ClFN₄O₂: C, 45.82; H, 3.20; N, 21.78%; Found: C, 45.84; H, 3.22; N, 21.76%.

General procedure for the synthesis of 5-fluoro-N⁴-(3-(nitrophenyl)-N²-(4-morpholino-phenyl)pyrimidine-2,4-diamine (4)

To a stirred solution of compound 3 (10 mmol), 4-morpholinobenzenamine (20 mmol) and K₂CO₃ (15 mmol) in dimethyl sulfoxide (10 mL). The reaction mixture was stirred at 90°C and followed by TLC. After completion of the reaction, the mixture was diluted and extracted with ethyl acetate solution. The organic layer was dried over anhydrous Na₂SO₄. Finally the compound was concentrated and recrystallized to get pure compound of 5-fluoro-N⁴-(3-(nitrophenyl)-N²-(4-morpholinophen-yl)pyrimidine-2,4-diamine as a pale yellow solid (4.26 g, 86%), m.p. 191-193°C; ¹H NMR (CDCl₃, 400 MHz): δ 8.23 (brs, 1H), 8.20 (brs, 1H), 7.76-7.68 (m, 2H), 7.40 (t, 1H, J=8.2 Hz), 7.36 (s, 1H), 7.21 (dd, 1H, J=7.05, 8.0 Hz), 6.86 (d, 2H, J=7.32 Hz), 6.78 (d, 2H, J=7.34 Hz), 3.90 (t, 4H, J=4.95 Hz), 3.27 (t, 4H, J=4.83 Hz); ¹³C NMR (CDCl₃, 75 MHz): δ 165.6, 154.4, 151.8, 148.5, 143.2, 140.4, 132.6, 132.2, 130.3, 123.2, 119.2, 115.3, 114.6, 110.8, 69.4, 51.8; ¹⁹F NMR (CDCl₃, 376 MHz): δ -118.31 (s, 1F); ESIMS: m/z 411 (M+H)⁺. Anal. Calcd. for C₂₀H₁₉FN₆O₃: C, 58.54; H, 3.65; N, 20.32%; Found: C, 58.50; H, 3.67; N, 20.36%.

General procedure for the synthesis of 5-fluoro-N⁴-(3-(aminophenyl)-N²-(4-morpholinophenyl)pyrimidine-2,4-diamine (5)

The compound 4 (10 mmol) in a mixture of methanol-ethyl acetate, (1:2, 20 mL) was treated with 10% Pd-carbon (5% w/w). The reaction was subjected to hydrogenation under 50 psi hydrogen gas pressures at room temperature and the reaction was monitored by TLC. After completion of the reaction, the mixture was filtered through a celite pad and concentrated under reduced pressure to afford pure product as a pale yellow solid (3.47 g, 78%), m.p. 205-206°C; ¹H NMR (CDCl₃, 400 MHz): δ 8.26 (brs, 1H), 8.20 (brs, 1H), 7.56-7.44 (m, 4H), 7.36 (t, 1H, J=7.2 Hz), 7.30 (s, 1H), 7.10 (dd, 1H, J=7.0, 8.20 Hz), 6.89 (d, 2H, J=7.30 Hz), 6.20 (s, 2H), 3.86 (t, 4H, J=4.89 Hz), 3.15 (t, 4H, J=4.78 Hz); ¹³C NMR (CDCl₃, 75 MHz): δ 165.8, 154.6, 151.4, 149.0, 143.2, 139.8, 132.6, 132.3, 130.4, 119.4, 115.0, 106.5, 102.4, 69.4, 51.8; ¹⁹F NMR (CDCl₃, 376 MHz): δ -118.33 (s, 1F); ESIMS: m/z 403 (M+Na)⁺. Anal. Calcd. for C₂₀H₂₁FN₆O: C, 63.27; H, 5.38; N, 22.82%; Found: C, 63.30; H, 5.35; N, 22.85%.

General procedure for the synthesis of 5-fluoro-N⁴-(3-(4-substitutedbenzylaminophenyl)-N²-(4-morpholinophenyl)pyrimidine-2,4-diamine derivatives (7a-J)

To a solution of compound 5 (10 mmol) in acetone 20 mL was added K₂CO₃ (25 mmol) at room temperature. This was followed by the addition of substituted benzyl halides 6 (12 mmol). The reaction mixture was heated under reflux for 3 h and the completion of reaction was checked by TLC. The reaction mixture was cooled to room temperature and poured into ice-water followed by extraction with ethyl acetate. The combined organic layer was evaporated, recrystallized to get pure compounds (7a-J) as solids.

5-Fluoro-N⁴-(3-(4-fluorobenzylamino)-phenyl)-N²-(4-morpholinophenyl)pyrimidine-2,4-diamine (7a)

Pale yellow solid (0.62 g, 91%), m.p. 238-240°C; ¹H NMR (DMSO-d₆, 400 MHz): δ 8.24 (brs, 1H), 8.22 (brs, 1H), 7.58-7.46 (m, 3H), 7.43 (s, 1H), 7.34 (d, 2H, J=8.54 Hz), 7.28-7.14 (m, 4H), 7.10 (t, 1H, J=10.0 Hz), 6.94 (d, 2H, J=7.84 Hz), 6.26 (brs, 1H), 4.42 (d, 2H, J=5.61 Hz), 3.82 (t, 4H, J=4.88 Hz), 3.28 (t, 4H, J=4.79 Hz); ¹³C NMR (DMSO-d₆, 75 MHz): δ 165.5, 161.4, 154.5, 151.2, 148.4, 143.8, 140.2, 137.4, 132.6, 132.3, 130.5, 128.8, 118.8, 115.2, 104.9, 103.7, 99.4, 69.2, 51.7, 46.3; ¹⁹F NMR (DMSO-d₆, 376 MHz): δ -118.30 (s, 1F); ESIMS: m/z 489 (M+H)⁺. Anal. Calcd. for C₂₇H₂₆F₂N₆O: C, 66.42; H, 5.36; N, 18.40%; Found: C, 66.38; H, 5.33; N, 18.38%.

5-Fluoro-N⁴-(3-(4-fluoro-2-(trifluoromethyl)benzylamino)-phenyl)-N²-(4-morpholinophenyl)pyrimidine-2,4-diamine (7b)

Colorless solid (0.81 g, 86%), m.p. 255-257°C; ¹H NMR (DMSO-d₆, 400 MHz): δ 8.34 (brs, 1H), 8.28 (brs, 1H), 7.62-7.56 (m, 4H), 7.42 (t, 1H, J=8.58 Hz), 7.36 (d, 2H, J=4.58 Hz), 7.28 (s, 1H), 7.20-7.12 (m, 2H), 6.98 (d, 2H, J=8.12 Hz), 6.28 (brs, 1H), 4.45 (d, 2H, J=5.58 Hz), 3.84 (t, 4H, J=4.86 Hz), 3.29 (t, 4H, J=4.82 Hz); ¹³C NMR (DMSO-d₆, 75 MHz): δ 165.7, 161.2, 155.0, 151.4, 148.6, 144.0, 140.3, 132.6, 132.3, 130.5, 128.9, 127.5, 121.8, 119.2, 118.8, 115.1, 112.2, 104.8, 103.5, 99.8, 69.4, 51.8, 46.4; ¹⁹F NMR (DMSO-d₆, 376 MHz): δ -118.27 (s, 1F), -122.39 (m, 3F); ESIMS: m/z 579 (M+Na)⁺. Anal. Calcd. for C₂₈H₂₅F₅N₆O: C, 58.63; H, 3.78; N, 14.98%; Found: C, 58.65; H, 3.75; N, 14.96%.

5-Fluoro-N⁴-(3-(3-fluorobenzylamino)-phenyl)-N²-(4-morpho-linophenyl)pyrimidine-2,4-diamine (7c)

Light brown solid (0.53 g, 80%), m.p. 223-224°C; ¹H NMR (CDCl₃, 400 MHz): δ 8.22 (brs, 1H), 8.19 (brs, 1H), 7.60-7.48 (m, 4H), 7.42 (d, 2H, J=8.60 Hz), 7.38 (s, 1H), 7.34-7.28 (m, 3H), 7.12 (t, 1H, J= 8.20 Hz), 6.98 (d, 2H, J=10.0 Hz), 6.27 (brs, 1H), 4.45 (d, 2H, J=5.62 Hz), 3.79 (t, 4H, J=4.76 Hz), 3.26 (t, 4H, J=4.81 Hz); ¹³C NMR (CDCl₃, 75 MHz): δ 165.5, 162.9, 154.4, 151.3, 148.6, 143.6, 139.8, 132.3, 132.1, 130.4, 122.7, 119.2, 115.1, 113.8, 113.5, 104.8, 103.6, 99.8, 69.3, 51.8, 46.3; ¹⁹F NMR (CDCl₃, 376 MHz): δ -118.19 (s, 1F); ESIMS: m/z 511 (M+Na)⁺. Anal. Calcd. for C₂₇H₂₆F₂N₆O: C, 66.36; H, 5.38; N, 18.38%; Found: C, 66.39; H, 5.36; N, 18.40%.

5-Fluoro-N⁴-(3-(4-(trifluoromethoxy)ben-zylamino)phenyl)-N²-(4-morpholinophenyl)pyri-midine-2,4-diamine (7d)

White solid (0.64 g, 83%), m.p. 260-262°C; ¹H NMR (CDCl₃, 400 MHz): δ 8.45 (brs, 1H), 8.38 (brs, 1H), 7.82-7.74 (m, 3H), 7.63 (s, 1H), 7.51 (d, 2H, J=10.0 Hz), 7.44-7.32 (m, 4H), 7.20 (t, 1H, J= 8.62 Hz), 7.10 (d, 2H, J=8.86 Hz), 6.32 (brs, 1H), 4.48 (d, 2H, J=5.80 Hz), 3.92 (t, 4H, J=4.72 Hz), 3.38 (t, 4H, J=4.89 Hz); ¹³C NMR (CDCl₃, 75 MHz): δ 165.5, 161.4, 155.2, 151.3, 148.5, 143.8, 139.8, 133.9, 132.5, 130.7, 128.0, 121.7, 119.3, 115.2, 114.0, 104.7, 103.5, 99.8, 69.6, 51.7, 46.2; ¹⁹F NMR (CDCl₃, 376 MHz): δ -118.32 (s, 1F), -123.84 (m, 3F); ESIMS: m/z 577 (M+Na)⁺. Anal. Calcd. for C₂₈H₂₆F₄N₆O₂: C, 58.80; H, 3.78; N, 16.08%; Found: C, 58.78; H, 3.75; N, 16.06%.

5-Fluoro-N⁴-(3-(2-fluoro-3-(trifluorometh-yl)benzylamino)-phenyl)-N²-(4-morpholinophen-yl) pyrimidine-2,4-diamine (7e)

Yellow solid (0.80 g, 92%), m.p. 245-247 °C; ¹H NMR (DMSO-d₆, 400 MHz): δ 8.42 (brs, 1H), 8.33 (brs, 1H), 7.90-7.82 (m, 4H), 7.71 (s, 1H), 7.63 (d, 2H, J=8.02 Hz), 7.40 (t, 1H, J=7.0 Hz), 7.36-7.28 (m, 2H), 6.97 (d, 2H, J=10.2 Hz), 6.35 (brs, 1H), 4.52 (d, 2H, J=5.84 Hz), 3.95 (t, 4H, J=4.82 Hz), 3.39 (t, 4H, J=4.90 Hz); ¹³C NMR (DMSO-d₆, 75 MHz): δ 164.8, 159.7, 155.3, 151.2, 148.7, 143.8, 139.6, 132.4, 132.2, 131.6, 130.3, 124.6, 124.4, 118.9, 118.5, 117.7, 115.1, 104.6, 103.3, 99.8, 69.5, 51.6, 44.6; ¹⁹F NMR (DMSO-d₆, 376 MHz): δ -118.29 (s, 1F), -122.86 (m, 3F); ESIMS: m/z 557 (M+H)⁺. Anal. Calcd. for C₂₈H₂₅F₅N₆O: C, 58.60; H, 3.75; N, 14.86%; Found: C, 58.63; H, 3.78; N, 14.88%.

5-Fluoro-N⁴-(3-(3-nitrobenzylamino)phen-yl)-N²-(4-morpholinophenyl)pyrimidine-2,4-diamine (7f)

Pale yellow solid (0.71 g, 85%), m.p. 234-235°C; ¹H NMR (CDCl₃, 400 MHz): δ 8.20 (brs, 1H), 8.17 (brs, 1H), 7.58-7.45 (m, 4H), 7.40 (d, 2H, J=10.0 Hz), 7.35 (s, 1H), 7.32-7.25 (m, 3H), 7.10 (t, 1H, J= 8.28 Hz), 6.96 (d, 2H, J=8.64 Hz), 6.25 (brs, 1H), 4.42 (d, 2H, J=5.64 Hz), 3.76 (t, 4H, J=4.52 Hz), 3.32 (t, 4H, J=4.75 Hz); ¹³C NMR (CDCl₃, 75 MHz): δ 164.5, 155.2, 151.0, 148.6, 147.5, 143.9, 142.6, 139.8, 133.6, 132.4, 130.5, 123.4, 121.9, 119.1, 115.0, 104.5, 103.4, 99.8, 69.2, 51.5, 45.2; ¹⁹F NMR (CDCl₃, 376 MHz): δ -118.27 (s, 1F); ESIMS: m/z 538 (M+Na)⁺. Anal. Calcd. for C₂₇H₂₆FN₆O₃: C, 62.80; H, 4.65; N, 18.78%; Found: C, 62.78; H, 4.62; N, 18.80%.

5-Fluoro-N⁴-(3-(3-methyl-4-nitrobenzyl-amino)phenyl)-N²-(4-morpholinophenyl)-pyrimidine-2,4-diamine (7g)

Color less solid (0.75 g, 87%) m.p. 217-219°C; ¹H NMR (CDCl₃, 400 MHz): δ 8.22 (brs, 1H), 8.14 (brs, 1H), 7.50-7.42 (m, 3H), 7.36 (d, 2H, J=8.0 Hz), 7.32 (s, 1H), 7.30-7.27 (m, 3H), 7.04 (t, 1H, J= 8.0 Hz), 6.90 (d, 2H, J=8.20 Hz), 6.28 (brs, 1H), 4.40 (d, 2H, J=5.60 Hz), 3.72 (t, 4H, J=4.52 Hz), 3.28 (t, 4H, J=4.80 Hz), 2.19 (s, 3H); ¹³C NMR (CDCl₃, 75 MHz): δ 164.3, 155.0, 149.8, 148.4, 147.5, 143.7, 142.2, 139.6, 132.2, 132.0, 130.4, 129.8, 124.9, 123.6, 119.0, 114.8, 104.3, 103.2, 99.7, 69.1, 51.3, 45.0, 19.8; ¹⁹F NMR (CDCl₃, 376 MHz): δ -118.28 (s, 1F); ESIMS: m/z 552 (M+Na)⁺. Anal. Calcd. for C₂₈H₂₈FN₆O₃: C, 62.84; H, 4.68; N, 20.50%; Found: C, 62.86; H, 4.65; N, 20.48%.

5-Fluoro-N⁴-(3-(4-fluoro-2-nitrobenzyl-amino)phenyl)-N²-(4-morpholinophenyl)pyri-midine-2,4-diamine (7h)

White solid (0.69 g, 85%), m.p. 280-282°C; ¹H NMR (CDCl₃, 400 MHz): δ 8.23 (brs, 1H), 8.19 (brs, 1H), 7.59-7.48 (m, 3H), 7.40 (s, 1H), 7.36 (d, 2H, J=8.56 Hz), 7.30-7.22 (m, 3H), 7.14 (t, 1H, J= 10.0 Hz), 6.98 (d, 2H, J=8.0 Hz), 6.28 (brs, 1H), 4.46 (d, 2H, J=5.74 Hz), 3.85 (t, 4H, J=4.80 Hz), 3.32 (t, 4H, J=4.46 Hz); ¹³C NMR (CDCl₃, 75 MHz): δ 164.5, 162.0, 155.5, 151.3, 148.9, 143.8, 139.6, 132.5, 132.3, 130.0, 129.4, 121.7, 121.5, 119.2, 115.1, 111.2, 104.5, 103.6, 99.9, 69.4, 51.8, 45.5; ¹⁹F NMR (CDCl₃, 376 MHz): δ -118.31 (s, 1F); ESIMS: m/z 556 (M+Na)⁺. Anal. Calcd. for C₂₇H₂₅F₂N₆O₃: C, 60.76; H, 3.82; N, 18.56%; Found: C, 60.78; H, 3.84; N, 18.54%.

2-((3-(5-Fluoro-2-(4-morpholinophenyl-amino)pyrimidin-4-ylamino)phenylamino)-methyl)-4-nitrophenol (7i)

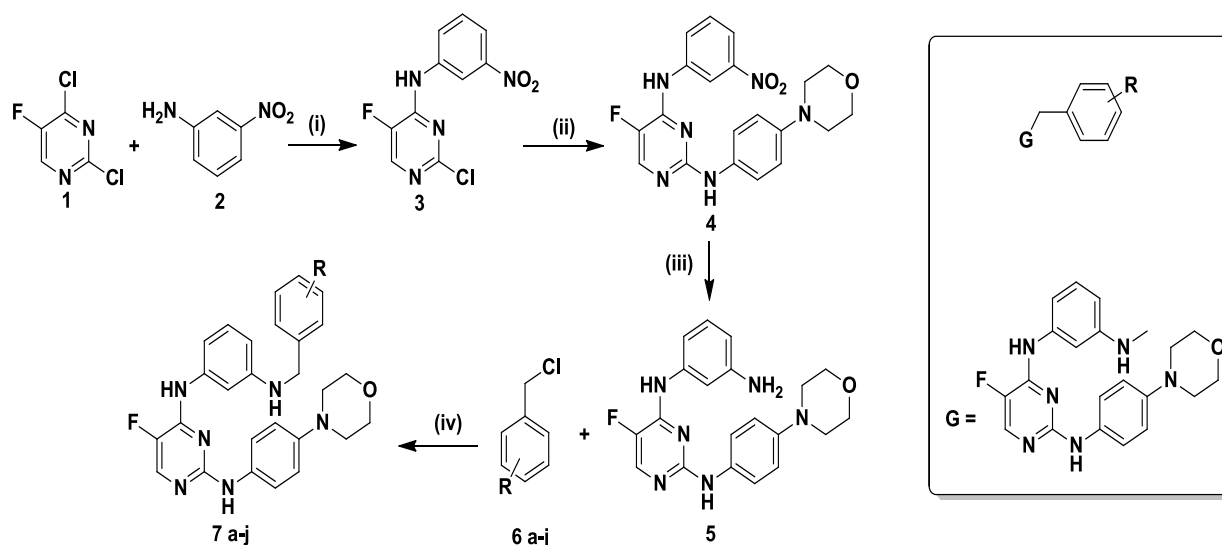
Light brown solid (0.59 g, 86%), m.p. 210-212°C; ¹H NMR (CDCl₃, 400 MHz): δ 10.48 (brs, 1H), 8.26 (brs, 1H), 8.20 (brs, 1H), 7.62-7.58 (m, 3H), 7.48 (d, 2H, J=10.0 Hz), 7.42 (s, 1H), 7.38-7.29 (m, 4H), 7.08 (t, 1H, J=8.0 Hz), 6.94 (d, 2H, J=8.0 Hz), 6.28 (brs, 1H), 4.46 (d, 2H, J=5.68 Hz), 3.82 (t, 4H, J=4.80 Hz), 3.38 (t, 4H, J=4.50 Hz); ¹³C NMR (CDCl₃, 75 MHz): δ 165.2, 160.5, 155.3, 151.6, 148.5, 143.9, 140.5, 139.7, 132.6, 132.3, 130.4, 129.8, 124.7, 123.5, 119.4, 116.7, 115.3, 104.8, 103.5, 99.8, 69.5, 51.8, 45.6; ¹⁹F NMR (CDCl₃, 376 MHz): δ -118.27 (s, 1F); ESIMS: m/z 554 (M+Na)⁺. Anal. Calcd. for C₂₇H₂₆FN₆O₄: C, 60.04; H, 3.96; N, 18.84%; Found: C, 60.07; H, 3.94; N, 18.86%.

5-Fluoro-N⁴-(3-(4-methoxybenzylamino)-phenyl) -N²-(4-morpholinophenyl)pyrimidine-2,4-diamine (7j)

Pale yellow solid (0.74 g, 78%), m.p. 218-220°C; ¹H NMR (CDCl₃, 400 MHz): δ 8.21 (brs, 1H), 8.18 (brs, 1H), 7.50-7.48 (m, 3H), 7.45 (s, 1H), 7.38 (d, 2H, J=8.0 Hz), 7.34-7.28 (m, 4H), 7.06 (t, 1H, J= 10.0 Hz), 6.85 (d, 2H, J=8.0 Hz), 6.25 (brs, 1H), 4.38 (d, 2H, J=5.80 Hz), 3.90 (s, 3H), 3.70 (t, 4H, J=4.80 Hz), 3.20 (t, 4H, J=4.88 Hz); ¹³C NMR (CDCl₃, 75 MHz): δ 164.5, 158.3, 155.2, 151.4, 148.6, 143.5, 139.7, 134.1, 132.5, 132.3, 130.6, 128.0, 118.8, 114.9, 114.3, 104.5, 103.3, 99.9, 69.2, 58.8, 46.2; ¹⁹F NMR (CDCl₃, 376 MHz): δ -118.34 (s, 1F); ESIMS: m/z 523 (M+Na)⁺. Anal. Calcd. for C₂₈H₂₉FN₆O₂: C, 68.42; H, 4.98; N, 16.86%; Found: C, 68.40; H, 4.96; N, 16.88%.

RESULTS AND DISCUSSION

The synthesis of 5-fluoro-*N*¹-(3-(4-substituted-benzylamino)phenyl)-*N*²-(4-morpholinophenyl) pyrimidine-2,4-diamine derivatives 7a-J were performed starting from the readily available 2,4-dichloro-5-fluoropyrimidine (1) and 3-nitroaniline (2). Compound 1 in the presence of K₂CO₃ and dimethyl sulfoxide medium was reacted with 3-nitroaniline 2 to give 2-chloro-5-fluoro-*N*-(3-nitrophenyl) pyrimidine-4-amine 3. Then pyrimidine 3 was treated with 4-morpholinobenzeneamine in the presence of K₂CO₃ to afford compound 4. The amino pyrimidine 5 was synthesized by the treatment of compound 4 with Pd/C in methanol/ethyl acetate mixture. The resulting amino pyrimidine 5 was converted into 5-fluoro-*N*¹-(3-(4-substitutedbenzylamino)phenyl)-*N*²-(4-morpholino-phenyl)pyrimidine-2,4-diamine derivatives 7a-J in a one-pot reaction employing nucleophilic substitution (S_N²) with various substituted benzyl halides 6a-J in the presence of K₂CO₃ in very good yields (Scheme 1). The structures of the newly synthesized compounds were determined based on their spectral ¹H-NMR, ¹³C-NMR, ¹⁹F-NMR, ESIMS and analytical data. The ¹H-NMR spectra of compound 3 showed a singlet at δ 7.43 ppm for pyrimidine -CH proton and a broad singlet at δ 8.24 ppm for -NH proton. The ¹H-NMR spectra of compound 4 exhibited two triplets for the -NCH₂, -OCH₂ groups at δ 3.27, δ 3.90 ppm, a singlet at δ 7.36 ppm due to pyrimidine -CH proton and two broad singlets appeared at δ 8.20, δ 8.23 ppm due to the two -NH protons. Similarly compound 5 showed two triplets at δ 3.15, δ 3.86 ppm due to -NCH₂, -OCH₂ groups, while the -NH₂ group protons appeared as a singlet at δ 6.20 ppm, two broad singlets appeared at δ 8.20, δ 8.26 ppm due to two -NH group protons respectively.



Scheme 1: Reagents and conditions: (i) K₂CO₃, DMSO, 90°C, 88% (ii) 4-morpholinobenzeneamine K₂CO₃, DMSO, 90°C, 86% (iii) 10% Pd/C, MeOH:EtOAc, room temperature, 78% (iv) substituted benzyl halides, K₂CO₃, acetone, reflux

BIOLOGICAL RESULTS

The primary means of controlling mosquito-borne diseases is by spraying insecticides. This led to insecticidal resistance and thus urged for the synthesis of new compounds having high potential activity. The compounds 7a-J synthesised in this protocol have proved to be promising agents showing impressive larvicidal activity. Mortality of larvae varied with different concentrations of compounds. The mortality of third instar larvae is indicated in Table 1. Among all the newly synthesized compounds, 7b showed highest mortality (91 ± 2.12) and reached to standard value at 100 ppm concentration at 72 hrs exposures, followed by 7d, 7e and 7h compounds. Impressive mortality was observed for, 7a, 7c, 7f, 7g and 7i compounds. The values 65 ± 1.12, 67 ± 3.24, 70 ± 2.78 mortality rates were considered as a least at 100 ppm concentration in 24, 48 and 72 hrs exposure of compound 7g respectively. The Structure-Activity Relationship (SAR) of compounds 7a-J was determined using the data presented in Table 1. SAR studies revealed that the presence of a strong electron-withdrawing group on the benzyl ring increases the anti-larvicidal activity. In particular, compounds with fluoro groups in either position 2 or position 4 of the benzyl ring demonstrated an enhanced potency against different panels of microbial strains.

The mortality results were identified as mortality in % ± Standard error and p value less than 0.05 was considered as significant. Corrected mortality was calculated with the Abbott's formula [26].

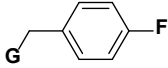
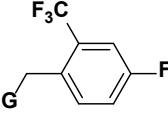
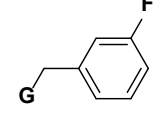
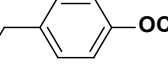
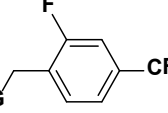
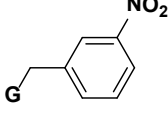
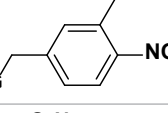
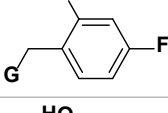
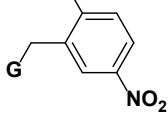
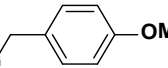
% of mortality = [Number of dead larvae / Number of larva introduced] × 100 Corrected mortality = Observed mortality in treatment - observed mortality in control × 100 / 100 - control mortality.

BIOLOGICAL ASSAY

Preparation of different concentrations of compound

One gram of each newly synthesized series of compounds 7a-J were taken in different tubes and mixed with 100 ml of distilled water to each tube and these were considered as the blank. Then different concentrations ranging from 50, 75 and 100 ppm were prepared

Table 1: Results of larvicidal activity of fluoro pyrimidine linked with morpholinophenyl, benzyl derivatives (7a-J)

Compound	R	Concentration (in ppm)	Period of exposure (h)		
			24	48	72
7a		-	24	48	72
		-	M ± S.E	M ± S.E	M ± S.E
		100	72 ± 3.39	74 ± 2.65	81 ± 3.12
		75	70 ± 3.75	71 ± 1.72	74 ± 3.54
		50	65 ± 1.61	66 ± 2.83	71 ± 1.74
7b		100	78 ± 1.45	84 ± 1.53	91 ± 2.12
		75	75 ± 1.11	79 ± 2.12	88 ± 1.91
		50	70 ± 1.56	72 ± 1.56	82 ± 2.23
7c		100	71 ± 1.49	73 ± 2.42	80 ± 3.41
		75	69 ± 1.76	70 ± 1.63	74 ± 3.41
		50	64 ± 1.46	65 ± 2.32	71 ± 1.43
7d		100	76 ± 3.33	78 ± 1.22	87 ± 1.84
		75	73 ± 2.68	74 ± 3.23	78 ± 1.49
		50	68 ± 1.55	69 ± 2.6	75 ± 1.63
7e		100	77 ± 2.22	81 ± 2.77	89 ± 2.67
		75	74 ± 1.89	76 ± 1.43	84 ± 2.86
		50	69 ± 2.71	70 ± 2.57	79 ± 1.93
7f		100	69 ± 1.27	71 ± 2.21	78 ± 2.61
		75	68 ± 1.65	69 ± 3.74	71 ± 2.68
		50	63 ± 3.49	64 ± 3.42	70 ± 3.4
7g		100	68 ± 1.12	70 ± 3.24	75 ± 2.78
		75	66 ± 1.27	68 ± 2.68	70 ± 2.57
		50	62 ± 2.26	63 ± 3.48	68 ± 1.46
7h		100	74 ± 2.42	77 ± 3.42	84 ± 3.46
		75	72 ± 1.61	73 ± 2.32	77 ± 2.72
		50	67 ± 3.62	68 ± 2.33	74 ± 2.21
7i		100	73 ± 1.52	75 ± 3.43	82 ± 1.48
		75	71 ± 1.64	72 ± 2.54	75 ± 2.81
		50	66 ± 2.86	67 ± 2.59	72 ± 1.53
7J		100	65 ± 3.36	67 ± 2.62	70 ± 3.35
		75	63 ± 2.49	64 ± 2.28	68 ± 2.27
		50	59 ± 2.54	61 ± 1.47	65 ± 2.46
Standard		25	100 ± 0.00	100 ± 0.00	100 ± 0.00

M=Mortality; SE=Standard Error

from the above stock. In contrast, 25 ppm of Malathion was prepared and it was considered as standard. All chemicals and reagents used were of analytical grade and obtained from Sigma.

Squito rearing

The larva were collected from Godavari area of Rajahmundry and confirmed by entomologist, Hyderabad. These larval forms were placed in plastic and enamel tray having 500 mL tap water and these larvae were feed with diet composed of brewer's yeast, dog biscuits and algal products in the ratio 3:1:1 respectively. Then the collected pupae were transferred into plastic bowl (12 × 12 cm) filled with water by taking the help of dipper. Later the plastic Jar was in mosquito cage (90 × 90 × 90 cm diameter) for adult's emergence. All these larval forms were maintained certain conditions such as 27 ± 2°C temperature, 75-85% relative humidity and 14:10 light and dark cycle. Before blood feeding, sugar solution (10% sucrose) was provided for 3 days. Adult female mosquitos were moved to feed on rabbit for blood (normally exposed site is dorsal side and 1 rabbit for day) continued up to 5 days for sufficient blood intake for development and growth. After completion of blood meal, that enamel water tray were moved to cage for oviposition. By following the method of Kamaraj *et al.* [27], all were maintained and reared in the lab.

Larvicidal bioassay

The larvicidal assay was done by the standard protocol of WHO with slight modifications [28]. Different concentrations (50, 75 and 100 ppm) of newly synthesized compounds (7a-J) were transferred in to glass petridish. In every batch 25 larvae of 3rd instar were transferred to the petri dish and 25 ppm concentration of Malathion was also transferred to petridish and exposed with larvae forms. Results were observed in every 24, 48, 72 hrs. The dead larvae were identified when they are failed to move after probing with needle in the siphon or cervical region. In this same way the experiment was carried out for 4 replicates simultaneously to all the concentrations. During the entire experimental period the larvae were unfed.

CONCLUSION

In conclusion, we have presented an efficient and benign synthesis of 5-fluoro benzyl amino pyrimidine systems containing morpholino-phenyl rings with excellent yields. All the synthesized compounds have been investigated for their larvicidal activity. From our newly synthesized compounds, it is seen that compounds 7b, 7d, 7e and 7h have shown excellent larvicidal activity. The activity of compounds 7a, 7c, 7f, 7g and 7i was very impressive. Accordingly, this novel class of new 5-fluoro-(4-substitutedbenzylamino)-(4-morpho-lylphenyl) pyrimidine derivatives emerged as a valuable lead series with great potential as larvicidal agents.

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