Synthesis, Biological Activity of Pyrimidine Linked with Morpholinophenyl Derivatives

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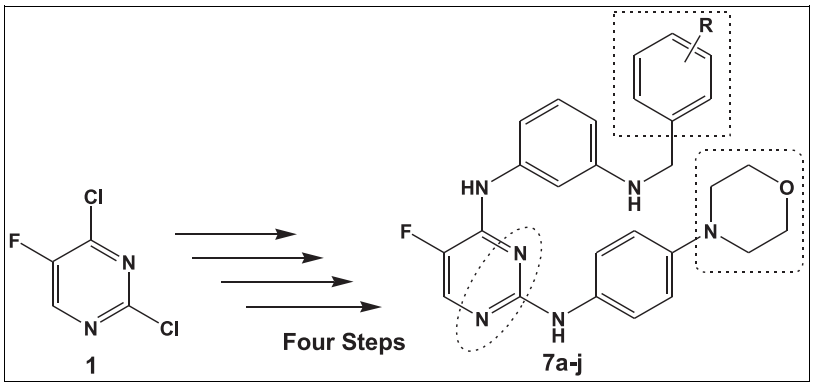


A new series of 5-fluoro-N^4-(3-(4-substitutedbenzylamino)phenyl)-N^2-(4-morpholinophenyl)pyrimidine-2,4-diamine derivatives (**7a–j**) are prepared from using an intermediate compound 5-fluoro-N^4-(3-(aminophenyl)-N^2-(4-morpholinophenyl)pyrimidine-2,4-diamine (**5**). The structures of the newly synthesized products are established from their spectral ^{1}H-NMR, ^{13}C-NMR, ^{19}F-NMR, ESI-MS, and analytical data. Here we report the synthesized compounds and larvicidal activity. All the compounds are screened for their significant larvicidal activity against third instar larvae at 24, 48, and 78-h time exposure, and values were compared with standard drug Malathion. The Compounds **7i, 7a, 7c, 7f,** and **7j** exhibited significant activity. However the compounds **7b, 7e, 7d,** and **7h** showed excellent activity when compared to the above compounds and to standard drug malathion too because of the presence of mild electron withdrawing groups such as trifluoro, fluorine, hydroxy, nitro, and methoxy derivatives which are attached to the benzyl ring.

INTRODUCTION

Mosquito-borne diseases, such as dengue fever, malaria, encephalitis, yellow fever, chikungunya, and filariasis (are among the most common/(or) have become one of the most dreadful) diseases, which are a major threat to millions of people spread 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* (*Ae. 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 [4]. In the last five years most of the insecticides did not show any effect on insects like mosquitoes because of increased drug resistance [5,6].

4-Morpholinophenyl derivatives represent an interesting class of compounds possessing a wide spectrum of biological activities. A large number of morpholinophenyl derivatives exhibited antibacterial [7,8], antifungal [9], anticonvulsant [10], analgesic [11], anticancer [12], and antiviral activity [13]. A number of types of pyrimidines were proved to possess valuable pharmacological properties, such as antiviral [14,15], antioxidant [16,17], antimalarials [18], anticonvulsant [19,20], Alzheimer's disease [21], antidepressant [22], antimicrobial [23,24], antitubercular [25,26], anti-inflammatory [27,28], antiplatelet [29], antibacterial [30], antifungal [31,32], anticancer agents [33,34], antiproliferative agent [35], and anti-HIV agents [14,36,37]. Integration of these moieties may show synergistic effect, and 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 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 anti-larvicidal screening of new derivatives (7a–j) with different pyrimidines and morpholinophenyl moiety pharmacophores as hybrid molecules possessing anti-larvicidal activity are investigated.

RESULTS AND DISCUSSION

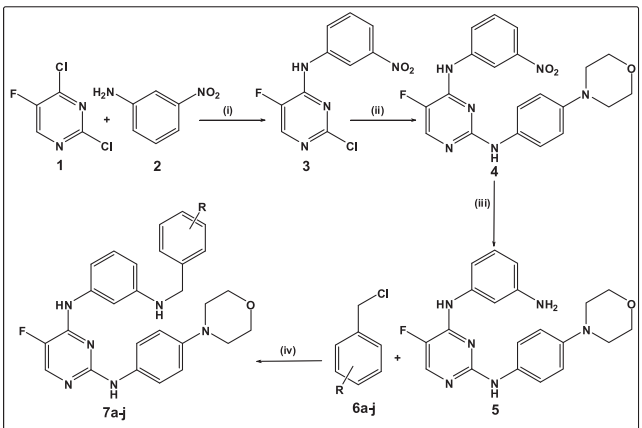
Chemistry. Compound **1** was treated with 3-nitrobenzenamine **2** in the presence of K_2CO_3 and dimethyl sulfoxide medium to give 2-chloro-5-fluoro-*N*-(3-nitrophenyl)pyrimidin-4-amine (**3**). The compound nitro pyrimidine (**3**) was reacted with 4-morpholinobenzenamine in the presence of K_2CO_3 as a basic media to afford compound (**4**) respectively. The aminopyrimidine compound (**5**) was performed by the treatment of compound **4** with Pd-Carbon in methanol-ethyl acetate solvent condition. Interestingly, the resultant amino pyrimidine **(5)** was further converted into 5-fluoro-N^4-(3-(4-substitutedbenzylamino)phenyl)-N^2-(4-morpholinophenyl)pyrimidine-2,4-diamine derivatives **(7a–j)** through one-pot reaction by dehydrohalogenation with various substituted benzyl halides **6a–j** in the presence of K_2CO_3 which is observed to give a very good yield (Scheme 1). The structures of the newly synthesized compounds were determined from their spectral ^{1}H-NMR, ^{13}C-NMR, ^{19}F-NMR, and ESIMS and analytical data.

All the synthesized compounds gave satisfactory analyses for the proposed structures, which were confirmed on the basis of their spectral data. The ^{1}H-NMR spectra of compound **(3)** showed peak at δ 7.43 ppm as a singlet for pyrimidine –CH proton and a broad singlet at δ 8.24 ppm for –NH proton. The ^{1}H-NMR spectra of the compound **(4)** exhibited two triplets for the $-NCH_2$, $-OCH_2$ groups at δ 3.27, δ 3.90 ppm, a singlet at δ 7.36 ppm because of pyrimidine –CH proton and two broad singlets appeared at δ 8.20, δ 8.23 ppm because of two –NH protons respectively. Similarly compound **(5)** showed two triplets at δ 3.15, δ 3.86 ppm because of $-NCH_2$, $-OCH_2$ groups, while the $-NH_2$ group protons appeared as a singlet at δ 6.20 ppm, two broad singlets appeared at δ 8.20, δ 8.26 ppm because of two –NH group protons respectively.

The structures of title compounds **(7a–j)** were assigned on the basis of their spectral studies. The physical data, ^{1}H-NMR, ^{13}C-NMR, ^{19}F-NMR, ESIMS, and analytical data for all the synthesized compounds are reported in experimental protocols.

Biological results. Insecticidal resistance is the main problem to control the mosquito population in the world. This is mainly achieved by synthesis of new compounds having high potential activity. The present study of anti-larvicidal activity indicated mortality of larvae varies from different concentrations of compounds. The percentages of mortality increased when increasing concentrations of the compounds. The mortality of third instar larvae was indicated in Table 1**.** Among all the newly synthesized compounds, **7b** showed highest

Scheme 1. Synthesis pathway for the pyrimidine linked with morpholinophenyl derivatives.



Compound	7a	7b	7c	7d	7e	7f	7g	7h	7i	7j
R	4-F	2-CF_3, 4-F	3-F	4-OCF_3	2-F, 4-CF_3	3-NO_2	3-Me, 4-NO_2	2-NO_2, 4-F	2-OH, 5-NO_2	4-OMe

Reagents & Conditions:
(i) K_2CO_3, DMSO, 90°C;
(ii) 4-morpholinobenzenamine, K_2CO_3, DMSO, 90°C;
(iii) 10% Pd/C, MeOH:EtOAc, rt;
(iv) substituted benzyl halides, K_2CO_3, acetone, reflux.

Table 1

Results of anti-larvicidal activity of fluoro pyrimidine linked with morpholinophenyl, benzyl derivatives (**7a–j**).

		Period of exposure		
		24	48	72
Compound	Concentration (in ppm)	M ± S.E	M ± S.E	M ± S.E
	100	72 ± 3.39	74 ± 2.65	81 ± 3.12
7a	75	70 ± 3.75	71 ± 1.72	74 ± 3.54
	50	65 ± 1.61	66 ± 2.83	71 ± 1.74
	100	78 ± 1.45	84 ± 1.53	91 ± 2.12
7b	75	75 ± 1.11	79 ± 2.12	88 ± 1.91
	50	70 ± 1.56	72 ± 1.56	82 ± 2.23
	100	71 ± 1.49	73 ± 2.42	80 ± 3.41
7c	75	69 ± 1.76	70 ± 1.63	74 ± 3.41
	50	64 ± 1.46	65 ± 2.32	71 ± 1.43
	100	76 ± 3.33	78 ± 1.22	87 ± 1.84
7d	75	73 ± 2.68	74 ± 3.23	78 ± 1.49
	50	68 ± 1.55	69 ± 26	75 ± 1.63
	100	77 ± 2.22	81 ± 2.77	89 ± 2.67
7e	75	74 ± 1.89	76 ± 1.43	84 ± 2.86
	50	69 ± 2.71	70 ± 2.57	79 ± 1.93
	100	69 ± 1.27	71 ± 2.21	78 ± 2.61
7f	75	68 ± 1.65	69 ± 3.74	71 ± 2.68
	50	63 ± 3.49	64 ± 3.42	70 ± 3.42
	100	65 ± 1.12	67 ± 3.24	70 ± 2.78
7g	75	63 ± 1.27	64 ± 2.68	68 ± 2.57
	50	59 ± 2.26	51 ± 3.48	65 ± 1.46
	100	74 ± 2.42	77 ± 3.42	84 ± 3.46
7h	75	72 ± 1.61	73 ± 2.32	77 ± 2.72
	50	67 ± 3.62	68 ± 2.33	74 ± 2.21
	100	73 ± 1.52	75 ± 3.43	82 ± 1.48
7i	75	71 ± 1.64	72 ± 2.54	75 ± 2.81
	50	66 ± 2.86	67 ± 2.59	72 ± 1.53
	100	68 ± 3.36	70 ± 2.62	75 ± 3.35
7j	75	66 ± 2.49	68 ± 2.28	70 ± 2.27
	50	62 ± 2.54	63 ± 1.47	68 ± 2.46
Malathion	25	100 ± 0.00	100 ± 0.00	100 ± 0.00

M = mortality; SE = standard error

mortality (91 ± 2.12) and reached to standard value at 100 ppm concentration at 72-h exposures, followed by **7e, 7d,** and **7h** compounds. Moderate mortality was observed by **7i, 7a, 7c, 7f,** and **7j** compounds. 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-h 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 increased the anti-larvicidal activity, and activity decreased in the presence of weak electron-withdrawing group or electron-releasing atoms or groups. Specifically, compounds with a trifluoro, fluoro groups in either position 2 or position 4 of the benzyl ring significantly increased potency against different panel of microbial strains. The dependence of compounds efficacy (biological activity) on the position of the fluoro group did not appear to be important. In this case, the use of electron withdrawing group on the benzyl ring in basic structures was worthy. Compounds bearing 4-F, 2-F, 4-CF_3, and 2-NO_2 exhibited more pronounced anti-larvicidal activity.

CONCLUSION

In conclusion, we have described efficient and benign synthesis of 5-fluoro benzylamino pyrimidine systems containing morpholinophenyl rings with excellent yields. All the synthesized compounds have been investigated for their anti-larvicidal activity. With our newly synthesized compounds, it is evident that, **7b 7e, 7d,** and **7h** compounds have exhibited high activity. Compounds **7i, 7a, 7c, 7f,** and **7j** have revealed excellent larvicidal activity. Accordingly, this novel class of new 5-fluoro-(4-substitutedbenzylamino)-(4-morpholinophenyl)pyrimidine derivatives which were reported from our laboratory, emerged as a valuable lead series with great potential as anti-larvicidal agents. This area of research may have a broad scope for the upcoming and very much promising researchers for further efficacy of evaluation.

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 F_{254} plates were used for thin layer chromatography (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 (^{1}H), 75 MHz (^{13}C), and 376 MHz (^{19}F) 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 Na_2SO_4.

General procedure for the synthesis of 2-chloro-5-fluoro-N-(3-nitrophenyl)pyrimidin-4-amine (3). 3-Nitrobenzenamine **2** (832 mg, 2 mmol) was added to a mixture of 2,4-dichloro-5-fluoropyrimidine **1** (500 mg, 1 mmol) and K_2CO_3 (625 mg, 1.5 mmol) in dimethyl sulfoxide (10 mL). The reaction mixture was stirred at 90°C for 3 h 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 Na_2SO_4, and was evaporated. The obtained compound was recrystallized with ethanol solvent to get 2-chloro-5-fluoro-N-(3-nitrophenyl)pyrimidin-4-amine as solid.

Yellow solid (0.432 g, 86%) m.p. 167–169°C (ethanol solvent used for crystallization); ^{1}H NMR ($CDCl_3$, 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); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 156.6, 155.5, 153.4, 147.6, 143.8, 141.8, 130.2, 122.8, 114.8, 111.7; ^{19}F NMR ($CDCl_3$, 376 MHz): δ −118.34 (s, 1F); ESIMS: *m/z* 291 $(M+Na)^+$. *Anal.* Cald. for $C_{10}H_6ClFN_4O_2$: 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-N^4-(3-(nitrophenyl)-N^2-(4-morpholinophenyl)pyrimidine-2,4-diamine (4). To a stirred solution of compound **3** (500 mg, 1 mmol), 4-morpholinobenzenamine (665 mg, 2 mmol) and K_2CO_3 (390 mg, 1.5 mmol) in dimethyl sulfoxide (10 mL). The reaction mixture was stirred for 3 h at 90°C. After completion of the reaction, the mixture was diluted and extracted with ethyl acetate solution. The organic layer was dried over anhydrous Na_2SO_4. Finally the compound was concentrated and recrystallized by ethanol to get pure solid compound of 5-fluoro-N^4-(3-(nitrophenyl)-N^2-(4-morpholinophenyl)pyrimidine-2,4-diamine.

Yellow solid (0.448 g, 85%) m.p. 191–193°C (ethanol solvent used for crystallization); ^{1}H NMR ($CDCl_3$, 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); ^{13}C NMR ($CDCl_3$, 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; ^{19}F NMR ($CDCl_3$, 376 MHz): δ −118.31 (s, 1F); ESIMS: *m/z* 411 $(M+H)^+$. *Anal.* Cald. for $C_{20}H_{19}FN_6O_3$: 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-N^4-(3-(aminophenyl)-N^2-(4-morpholino--phenylpyrimidine-2,4-diamine (5). The compound **4** (500 mg, 1 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 for 2.5 h, 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 in a good yield.

Yellow solid (0.410 g, 79%) m.p. 205–206°C (ethanol solvent used for crystallization); ^{1}H NMR ($CDCl_3$, 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); ^{13}C NMR ($CDCl_3$, 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; ^{19}F NMR ($CDCl_3$, 376 MHz): δ −118.33 (s, 1F); ESIMS: *m/z* 403 $(M+Na)^+$. *Anal.* Cald. for $C_{20}H_{21}FN_6O$: 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-N^4-(3-(4-substitutedbenzylamino)--phenyl)-N^2-(4-morpholinophenyl) pyrimidine-2,4-diamine derivatives (7a–j). To a solution of compound **5** (100 mg, 1 mmol) in acetone 20 mL was added K_2CO_3 (155 mg, 2.5 mmol) at room temperature. This was followed by the addition of substituted benzyl halides **6** (180 mg, 1.2 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 with ethanol to get pure compounds (**7a–j**) as solids.

5-Fluoro-N^4-(3-(4-fluorobenzylamino)phenyl)-N^2-(4-morpholinophenyl)pyrimidine-2,4-diamine (7a). Yellow solid (0.091 g, 91%) m.p. 238–240°C (ethanol solvent used for crystallization); ^{1}H NMR (DMSO–d_6, 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); ^{13}C NMR (DMSO–d_6, 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; ^{19}F NMR (DMSO–d_6, 376 MHz): δ −118.30 (s, 1F); ESIMS: *m/z* 489 $(M+H)^+$. *Anal.* Cald. for

$C_{27}H_{26}F_2N_6O$: 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). Light yellow solid (0.074 g, 76%) m.p. 255–257°C (ethanol solvent used for crystallization); ^{1}H NMR (DMSO–d_6, 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); ^{13}C NMR (DMSO–d_6, 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; ^{19}F NMR (DMSO–d_6, 376 MHz): δ −118.27 (s, 1F), −122.39 (m, 3F); ESIMS: *m/z* 579 (M +Na)$^+$. *Anal.* Cald. for $C_{28}H_{25}F_5N_6O$: 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-morpholinophenyl)pyrimidine-2,4-diamine (7c). Yellow solid (0.077 g, 78%) m.p. 223–224°C (ethanol solvent used for crystallization); ^{1}H NMR ($CDCl_3$, 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); ^{13}C NMR ($CDCl_3$, 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; ^{19}F NMR ($CDCl_3$, 376 MHz): δ −118.19 (s, 1F); ESIMS: *m/z* 511 (M + Na)$^+$. *Anal.* Cald. for $C_{27}H_{26}F_2N_6O$: 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)benzylamino)phenyl)-N²-(4-morpholinophenyl)pyrimidine-2,4-diamine (7d). Light yellow solid (0.079 g, 80%) m.p. 260–262°C (ethanol solvent used for crystallization); ^{1}H NMR ($CDCl_3$, 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); ^{13}C NMR ($CDCl_3$, 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; ^{19}F NMR ($CDCl_3$, 376 MHz): δ −118.32 (s, 1F), −123.84 (m, 3F); ESIMS: *m/z* 577 (M+Na)$^+$. *Anal.* Cald. for $C_{28}H_{26}F_4N_6O_2$: 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-(trifluoromethyl)benzylamino)phenyl)-N²-(4-morpholinophenyl)--pyrimidine-2,4-diamine (7e). White solid (0.091 g, 92%) m.p. 245–247°C (ethanol solvent used for crystallization); ^{1}H NMR (DMSO–d_6, 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); ^{13}C NMR (DMSO–d_6, 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; ^{19}F NMR (DMSO–d_6, 376 MHz): δ −118.29 (s, 1F), −122.86 (m, 3F); ESIMS: *m/z* 557 (M+H)$^+$. *Anal.* Cald. for $C_{28}H_{25}F_5N_6O$: 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)phenyl)-N²-(4-morpholinophenyl)pyrimidine-2,4-diamine (7f). Pale yellow solid (0.085 g, 85%) m.p. 234–235°C (ethanol solvent used for crystallization); ^{1}H NMR ($CDCl_3$, 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); ^{13}C NMR ($CDCl_3$, 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; ^{19}F NMR ($CDCl_3$, 376 MHz): δ −118.27 (s, 1F); ESIMS: *m/z* 538 (M+Na)$^+$. *Anal.* Cald. for $C_{27}H_{26}FN_7O_3$: 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-nitrobenzylamino)phenyl)-N²-(4-morpholinophenyl)pyrimidine-2,4--diamine (7g). White solid (0.087 g, 88%) m.p. 217–219°C (ethanol solvent used for crystallization); ^{1}H NMR ($CDCl_3$, 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); ^{13}C NMR ($CDCl_3$, 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; ^{19}F NMR ($CDCl_3$, 376 MHz): δ −118.28 (s, 1F); ESIMS: *m/z* 552 (M +Na)$^+$. *Anal.* Cald. for $C_{28}H_{28}FN_7O_3$: 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-nitrobenzylamino)phenyl)-N²-(4-morpholinophenyl)pyrimidine--2,4-diamine (7h). Yellow solid (0.077 g, 78%) m.p. 280–282°C (ethanol solvent used for crystallization); ^{1}H NMR ($CDCl_3$, 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); ^{13}C NMR ($CDCl_3$, 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; ^{19}F NMR ($CDCl_3$, 376 MHz): δ −118.31 (s, 1F); ESIMS: *m/z* 556 (M+Na)$^+$. *Anal.* Cald. for $C_{27}H_{25}F_2N_7O_3$: 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-morpholinophenylamino)pyrimidin-4-ylamino)phenylamino)methyl)-4-nitrophenol (7i). Pale yellow solid (0.089 g, 90%) m.p. 210–212°C (ethanol solvent used for crystallization); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 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); ^{13}C NMR ($CDCl_3$, 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; ^{19}F NMR ($CDCl_3$, 376 MHz): δ −118.27 (s, 1F); ESIMS: *m/z* 554 $(M+Na)^+$. *Anal.* Cald. for $C_{27}H_{26}FN_7O_4$: C, 60.04; H, 3.96; N, 18.84%; Found: C, 60.07; H, 3.94; N, 18.86%.

5-Fluoro-N^4-(3-(4-methoxybenzylamino)phenyl)-N^2-(4-morpholinophenyl)pyrimidine-2,4--diamine (7j). Yellow solid (0.080 g, 81%) m.p. 218–220°C (ethanol solvent used for crystallization); ^{1}H NMR ($CDCl_3$, 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); ^{13}C NMR ($CDCl_3$, 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; ^{19}F NMR ($CDCl_3$, 376 MHz): δ −118.34 (s, 1F); ESIMS: *m/z* 523 $(M+Na)^+$. *Anal.* Cald. for $C_{28}H_{29}FN_6O_2$: C, 68.42; H, 4.98; N, 16.86%; Found: C, 68.40; H, 4.96; N, 16.88%.

Biological assay. ***Preparation of different concentrations of compound.*** One gram of each newly synthesized series of compounds **7a–j** was 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 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. [38], all were maintained and reared in the lab.

Larvicidal bioassay. The larvicidal assay was done by the standard protocol of WHO with slight modifications [39]. 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, and 72 h. 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. 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 [40].

$$\%\ \text{of mortality} = [\text{Number of dead larvae}/\text{Number of larva introduced}] \times 100.$$

$$\text{Corrected mortality} = \text{Observed mortality in treatment} - \text{observed mortality in control} \times 100/100 - \text{control mortality}.$$

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