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Original article

Synthesis and *in vitro* microbiological evaluation of an array of biolabile 2-morpholino-*N*-(4,6-diarylpyrimidin-2-yl)acetamides

V. Kanagarajan, J. Thanusu, M. Gopalakrishnan*

Synthetic Organic Chemistry Laboratory, Department of Chemistry, Annamalai University, Annamalainagar 608 002, Tamil Nadu, India

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ABSTRACT

Biolabile 2-morpholino-*N*-(4,6-diarylpyrimidin-2-yl)acetamides **34**–**42** have been synthesized and evaluated for their *in vitro* antibacterial and antifungal activities. The minimum inhibitory concentration tested for the same compounds against the same set of bacterial and fungal strains shows that the compounds **36** and **38** against *β-Heamolytic streptococcus* and *Klebsiella pneumonia*, **40** against *Escherichia coli* and *Pseudomonas*, have excellent antibacterial activity. Compounds **36**, **38** and **42** show inhibition against *Aspergillus flavus*, compound **41** against *Microsporum gypsuem*, **42** against *Mucor*, and compounds **39** and **40** against *Rhizopus*.

1. Introduction

Now-a-days, there has been a growing interest pertaining to the synthesis of bioactive compounds in the field of organic chemistry. Among the nitrogen containing heterocyclic compounds, pyrimidines apparently gained considerable importance owing to their varied biological properties and therapeutical importance. Pyrimidines are the basic nucleus in nucleic acids and have been associated with a number of biological activities. Substituted aminopyrimidine nuclei are common in marketed drugs such as anti-atherosclerotic aronixil®, anti-histaminic thonzylamine®, anti-anxielytic buspirone®, anti-psoriatic enazadrem®, and other medicinally relevant compounds. Some notable biological activity of pyrimidine derivatives includes adenosine receptor antagonists [1], kinase inhibitors [2], analgesic [3], anti-inflammatory [3], inhibitors of cyclin-dependent kinases 1 and 2 [4], calcium channel antagonist [5], anti-histaminic [6], antitubercular [7] activities.

Promising diverse pharmacological activities were shown by various N-fuctionalized morpholines. They were reported to exert a number of important physiological activities such as antidiabetic [8], antiemetic [9], platelet aggregation inhibitors, anti-hyperlipoproteinemics [8] bronchodilators, growth stimulants [10] and antidepressants [11]. These were also used in the treatment of inflammatory diseases, pain, migraine and asthma [12]. Tridemorph®, a morpholine derivative was used as an antifungal agent [13]. 4-Phenyl morpholine derivatives were reported to possess anti-inflammatory [14] and central nervous system [15] activities. Besides these, amides are well known for their therapeutic values. The chemistry of amides having a chloroacetyl group was also very fascinating and has received significant attention now-a-days. *N*-Benzyl-β-chloropropionamide was a well-proven anticonvulsant agent [16]. Moreover, synthesis and *in vitro* antimicrobial activity of a new heterocyclic compounds which contain both morpholine and pyrimidine moiety together namely 4-(4-morpholinophenyl)-6-arylpyrimidin-2-amines were reported [17].

It was known from Scheme 1 that some clinically useful compounds containing pyrimidines moiety exhibit strong tuberculosis (A) and antimicrobial activity (B). Besides, some of the clinically important drugs contain morpholine moiety in addition to N-heterocycles which are separated by one or higher number of carbon atoms. Drugs derived from morpholine incorporated compounds include dextromoramide®, (C) a narcotic analgesic and doxapram HCl, (D) a respiratory stimulant. Doxapram® was used in the treatment of respiratory depression following anaesthesia. *N*-Benzyl-β-chloropropionamide (E) was a very good anticonvulsant and was marketed under the trade name Hibicon® and Hydrane®.

Recently, we exploited the synthesis of 3,4-dihydropyrimidin-2(1*H*)-ones [18], 2-phenyl-3-(4,6-diarylpyrimidin-2-yl)thiazolidin-4-ones [19], 6-aryl-1,2,4,5-tetrazinane-3-thiones [20], 2,6-

* Corresponding author. Tel.: +91 4144 228 233.
E-mail address: profmgk@yahoo.co.in (M. Gopalakrishnan).

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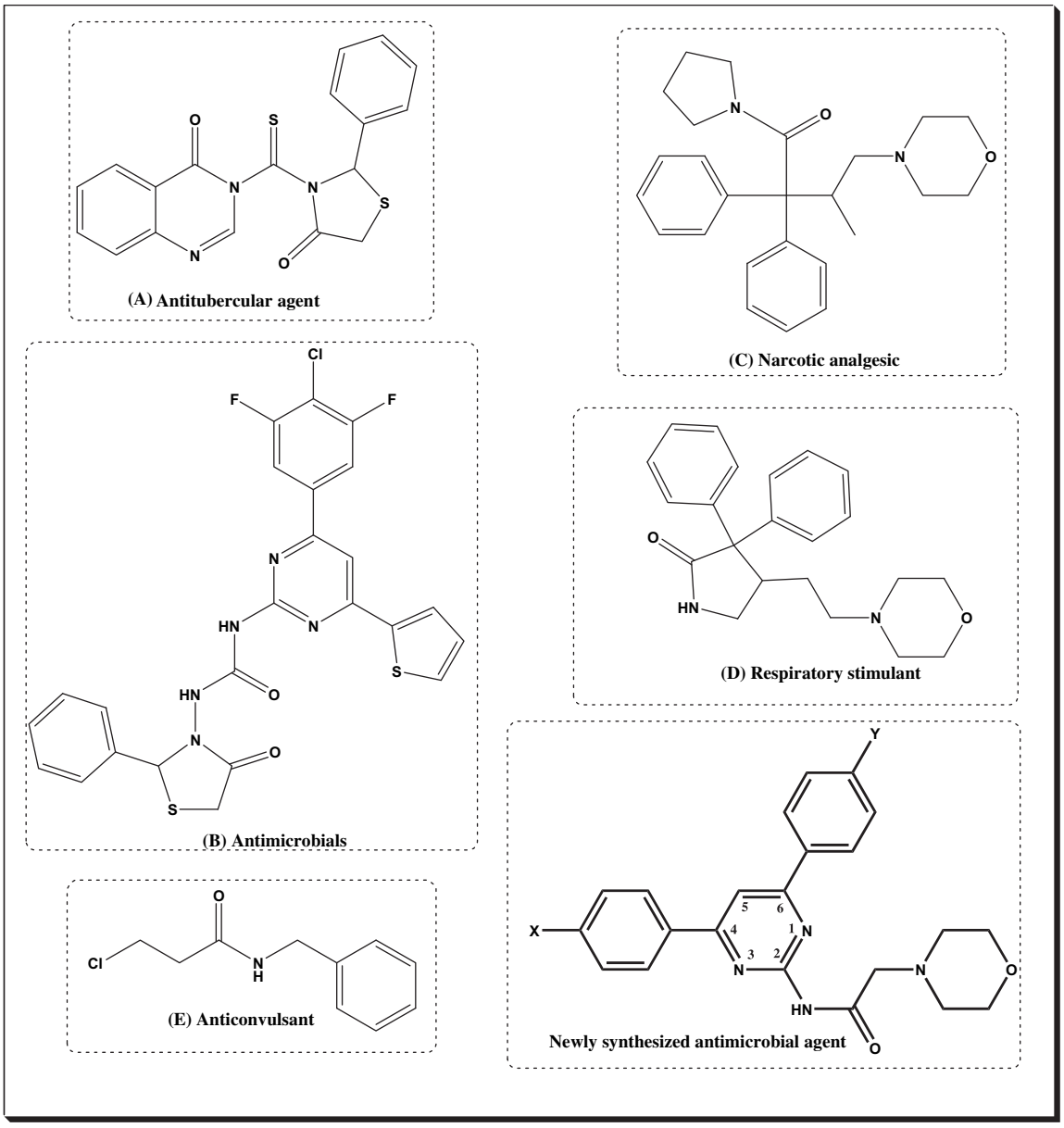


Scheme 1. Some of the synthetic compounds having the core pyrimidine, acetamide and morpholine nuclei with therapeutic activities.

diarylpiperidin-4-one derivatives [21-23] with a view to incorporate various other bioactive heterocyclic nucleus such as 1,2,3-selenadiazoles, 1,2,3-thiadiazoles, diazepans intact for evaluation of associated antibacterial and antifungal activities. Commercially available drugs have either pyrimidine or morpholine moiety only. In continuation of our earlier work on the synthesis of various structurally diverse heterocyclic compounds, we thought it was worthwhile to synthesize compounds comprising all pyrimidines, chloroacetyl amides and morpholine moiety together to furnish a compact structure like title 2-morpholino-*N*-(4,6-diarylpyrimidin-2-yl)acetamides **34**–**42** with the hope to develop some promising antimicrobial agents.

2. Results and discussion

2.1. Chemistry

The Claisen–Schmidt condensation of equimolar quantities of appropriate acetophenone and appropriate benzaldehyde in the presence of sodium hydroxide yielded *E*-1,3-diarylprop-2-en-1-ones **7**–**15**. When *E*-1,3-diarylprop-2-en-1-ones **7**–**15** were refluxed with guanidine nitrate in the presence of sodium hydroxide, 2-amino-4,6-diarylpyrimidines **16**–**24** were formed. Various substituted 2-chloro-*N*-(4,6-diarylpyrimidin-2-yl)acetamides **25**–**33** were synthesized by electrophilic substitution reaction of chloroacetyl chloride with the corresponding parent 2-amino-4,6-diarylpyrimidines **16**–**24** in the presence of triethylamine as base and toluene as solvent. Besides using sodium carbonate/potassium carbonate as a base and toluene as solvent, appreciable yields were obtained when triethylamine was used as a base and toluene as solvent to effect chloroacetylation of 2-amino-4,6-diarylpyrimidines at ambient temperature. In addition, while using stronger bases as sodium hydroxide, potassium hydroxide and pyridine individually to effect chloroacetylation, undesired products were obtained along with the expected product. The physical and analytical data for compounds **7**–**33** was given in Table 1. Then, condensation of 2-chloro-*N*-(4,6-diarylpyrimidin-2-yl)acetamides **25**–**33** with morpholine in the presence of anhydrous potassium

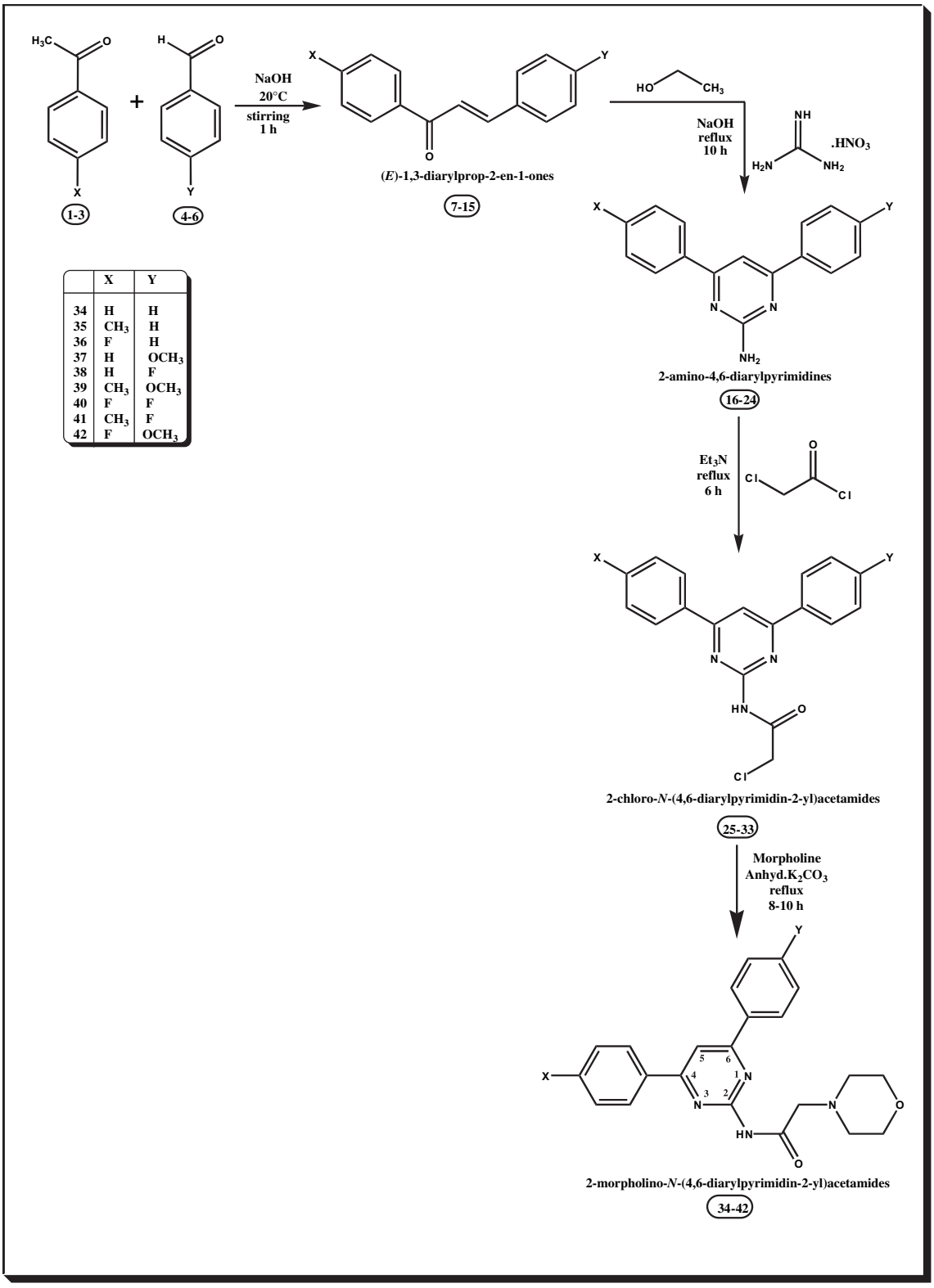
Table 1
Physical and analytical data of intermediate compounds **7**–**33**.

Compounds	m/z $(M+H)^+$ / Molecular formula	m.p. (°C)	Yield (%)	IR frequencies (cm^{-1})
7	209 $C_{15}H_{12}O$	52	96	3059, 2925, 2847, 1659, 1602, 689, 746
8	223 $C_{16}H_{14}O$	46	92	3025, 2972, 2916, 2858, 1655, 1605, 822, 692, 669
9	227 $C_{15}H_{11}FO$	58	90	3060, 3027, 2923, 2847, 1662, 1602, 1033, 764, 609
10	239 $C_{16}H_{14}O_2$	65	94	3057, 3013, 2949, 2907, 2841, 1656, 1598, 822, 687, 776
11	227 $C_{15}H_{11}FO$	73	98	3064, 3033, 2934, 1659, 1596, 1011, 776, 687
12	253 $C_{17}H_{16}O_2$	82	90	3071, 3033, 2965, 2933, 2839, 1653, 1605, 1033, 815, 736, 678
13	245 $C_{15}H_{10}F_2O$	90	95	3073, 3011, 2934, 1659, 1605, 1019, 742, 666
14	241 $C_{16}H_{13}FO$	98	85	3065, 3046, 2916, 2858, 1654, 1604, 1025, 818, 737, 673
15	257 $C_{16}H_{13}FO_2$	82	88	3071, 3022, 2983, 2940, 2841, 1659, 1600, 1018, 815, 742, 668
16	248 $C_{16}H_{13}N_3$	120	86	3478, 3303, 3184, 3062, 2967, 1629, 1568, 1364, 1225, 763, 697
17	262 $C_{17}H_{15}N_3$	66	80	3487, 3312, 3197, 3063, 2919, 3035, 1633, 1568, 1363, 1219, 819, 768, 697
18	266 $C_{16}H_{12}FN_3$	65	85	3495, 3329, 3201, 3053, 2924, 1637, 1568, 1361, 1225, 1020, 766, 694
19	278 $C_{17}H_{15}N_3O$	134	80	3366, 3325, 3199, 3005, 2972, 2934, 1643, 1565, 1360, 1240, 822, 772, 686
20	266 $C_{16}H_{12}FN_3$	101	85	3495, 3328, 3201, 3050, 2983, 2927, 1637, 1569, 1360, 1225, 1044, 766, 692
21	292 $C_{18}H_{17}N_3O$	52	85	3466, 3287, 3171, 3057, 2995, 2964, 2924, 1635, 1577, 1364, 1248, 1032, 816, 673, 582
22	284 $C_{16}H_{11}F_2N_3$	89	85	3494, 3330, 3210, 3068, 2975, 2923, 1642, 1573, 1365, 1299, 1015, 565, 503
23	280 $C_{17}H_{14}FN_3$	84	80	3497, 3315, 3195, 3073, 3041, 2920, 1638, 1574, 1361, 1227, 1016, 803, 668, 568
24	296 $C_{17}H_{14}FN_3O$	110	80	3478, 3318, 3203, 3053, 3003, 2927, 1634, 1572, 1369, 1299, 1036, 816, 665, 571
25	324 $C_{18}H_{14}ClN_3O$	110	65	3391, 3194, 3055, 2923, 2849, 1671, 1610, 1561, 1361, 1227, 837, 759, 689
26	338 $C_{19}H_{16}ClN_3O$	98	65	3358, 3296, 3200, 3150, 3058, 3035, 2958, 2919, 2851, 1681, 1594, 1530, 1339, 1203, 818, 815, 768, 691
27	342 $C_{18}H_{13}ClFN_3O$	202	50	3397, 3268, 3150, 3079, 3008, 2953, 2920, 2851, 1681, 1597, 1512, 1342, 1233, 1023, 840, 768, 689
28	354 $C_{19}H_{16}ClN_3O_2$	116	60	3393, 3276, 3073, 3008, 2953, 2922, 2851, 1681, 1595, 1525, 1342, 1249, 833, 830, 768, 687
29	342 $C_{18}H_{13}ClFN_3O$	81	60	3386, 3260, 3112, 3064, 2953, 2921, 2851, 1681, 1599, 1509, 1358, 1229, 1018, 837, 767, 694
30	368 $C_{20}H_{18}ClN_3O_2$	106	60	3328, 3002, 2958, 2920, 2848, 1682, 1591, 1513, 1336, 1248, 1025, 818, 814, 781, 634
31	360 $C_{18}H_{12}ClF_2N_3O$	204	45	3339, 3271, 3079, 2980, 2958, 2919, 2851, 1676, 1600, 1510, 1343, 1234, 1013, 834, 782, 635
32	356 $C_{19}H_{15}ClFN_3O$	85	55	3214, 3068, 3030, 2953, 2920, 2852, 1682, 1598, 1509, 1357, 1232, 1017, 843, 817, 777, 684
33	372 $C_{19}H_{15}ClFN_3O_2$	206	55	3342, 3289, 3161, 3073, 2997, 2958, 2918, 2848, 1681, 1598, 1524, 1340, 1238, 831, 828, 1034, 701, 631

carbonate furnished 2-morpholino-*N*-(4,6-diarylpyrimidin-2-yl)-acetamides **34**–**42**. Thus a four-step synthetic route furnished the target compounds 2-morpholino-*N*-(4,6-diarylpyrimidin-2-yl)acetamides **34**–**42** in good yields. A general schematic representation was given in Scheme 2. The physical and analytical data for compounds **34**–**42** was given in Table 2. The structures of all the newly synthesized compounds were supported by help of m.p.'s, elemental analysis, FT-IR, MS, one-dimensional NMR (^1H, ^{13}C) spectra.

2.2. Antibacterial activity

Novel 2-morpholino-*N*-(4,6-diarylpyrimidin-2-yl)acetamides **34**–**42** were tested for their antibacterial activity *in vitro* against *Staphylococcus aureus*, *β-Heamolytic streptococcus*, *Vibreo cholerae*, *Escherichia coli*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa*. Ciprofloxacin was used as standard drug. Minimum inhibitory concentration (MIC) in μg/mL values was reproduced in Table 3. Of the nine compounds **34**–**42** tested for their antibacterial activity by two-fold serial dilution method, compound **34** which has no substitution at the *para* position of phenyl rings attached to C-4 and C-6 carbons of pyrimidine moiety did not promote much activity against the tested bacterial strains, whereas compounds **35** and **37** which have electron donating $-CH_3$ and $-OCH_3$ groups at the *para* position of the phenyl ring exhibit moderate activity. The introduction of *p*-fluoro substituted phenyl rings in place of phenyl ring was found to enhance the antibacterial potency significantly. Thus the compounds **36** and **38** against *β-H. streptococcus* and



	X	Y
34	H	H
35	CH_3	H
36	F	H
37	H	OCH_3
38	H	F
39	CH_3	OCH_3
40	F	F
41	CH_3	F
42	F	OCH_3

Scheme 2. Synthesis of 2-morpholino-*N*-(4,6-diarylpyrimidin-2-yl)acetamides.

K. pneumonia, while **40** against *E. coli* and *Pseudomonas* show excellent antibacterial activity by inhibiting the growth of the respective organisms at a minimum inhibitory concentration of 6.25 μg/mL.

Moreover, compound **39** having electron donating $-CH_3$ group at the *para* position of the phenyl ring at C-4 carbon of pyrimidine and $-OCH_3$ group at the *para* position of the phenyl ring at C-6 carbon of pyrimidine exerted antibacterial activity against *V. cholerae* at 12.5 μg/mL MIC. Compounds **35** and **37** which have respectively $-CH_3$ and $-OCH_3$ groups at the *para* position of the phenyl rings at C-4/C-6 positions of the pyrimidine respectively did not have high activity against *V. cholerae*. Conversely compound **39**

Table 2
Physical and analytical data of 2-morpholino-*N*-(4,6-diarylpyrimidin-2-yl)acetamides **34**–**42**.

Compounds	X	Y	Yield (%)	m.p. (°C)	Elemental analysis (%)			m/z $(M+H)^{+\cdot}$
					C found (calculated)	H found (calculated)	N found (calculated)	Molecular formula
34	H	H	58	45	70.55 (70.57)	5.88 (5.92)	14.90 (14.96)	375 $C_{22}H_{22}N_4O_2$
35	CH_3	H	44	40	71.06 (71.11)	6.20 (6.23)	14.38 (14.42)	389 $C_{23}H_{24}N_4O_2$
36	F	H	45	50	67.30 (67.33)	5.35 (5.39)	14.25 (14.28)	393 $C_{22}H_{21}FN_4O_2$
37	H	OCH_3	88	40	68.28 (68.30)	5.95 (5.98)	13.82 (13.85)	405 $C_{23}H_{24}N_4O_3$
38	H	F	76	45	67.30 (67.33)	5.36 (5.39)	14.25 (14.28)	393 $C_{22}H_{21}FN_4O_2$
39	CH_3	OCH_3	54	50	68.87 (68.88)	6.22 (6.26)	13.36 (13.39)	419 $C_{24}H_{26}N_4O_3$
40	F	F	41	45	64.31 (64.38)	4.87 (4.91)	13.62 (13.65)	411 $C_{22}H_{20}F_2N_4O_2$
41	CH_3	F	48	55	67.95 (67.97)	5.66 (5.70)	13.76 (13.78)	407 $C_{23}H_{23}FN_4O_2$
42	F	OCH_3	50	50	65.36 (65.39)	5.46 (5.49)	13.21 (13.26)	423 $C_{23}H_{23}FN_4O_3$

which has both the $-CH_3$ and $-OCH_3$ groups at the above mentioned positions exerted potent activity against *V. cholerae*. Compound **41** (*p*-CH_3/F – substituents in the phenyl ring at C-4 and C-6 of pyrimidine) against *S. aureus* and *V. cholerae* and compound **42** (*p*-F/OCH_3 – substituents in the phenyl ring at C-4 and C-6 of pyrimidine) against *Pseudomonas* have exerted strong activity at an MIC value of 12.5 μg/mL and 6.25 μg/mL respectively.

2.3. Antifungal activity

The *in vitro* antifungal activity of 2-morpholino-*N*-(4,6-diarylpyrimidin-2-yl)acetamides **34**–**42** was studied against the fungal strains *viz.*, *Aspergillus flavus*, *Mucor*, *Rhizopus* and *Microsporum gypsuem*. Fluconazole was used as a standard drug. MIC in μg/mL values was reproduced in Table 3. Similar to antifungal activity, compound **34** which has no substitution at the *para* position of phenyl rings attached to C-4 and C-6 carbons of pyrimidine moiety did not promote much activity against the tested fungal strains, whereas compounds **35** and **37** which have electron donating $-CH_3$ and $-OCH_3$ groups at the *para* position of the phenyl rings exhibit moderate activity. Compounds **36** and **38**, both have electron withdrawing fluoro group in the phenyl rings and **42**, which have either the fluoro and the methoxy groups at the *para* positions of the phenyl rings at C-4 and C-6 positions of the pyrimidine respectively were more potent against *A. flavus* (MIC value = 6.25 μg/mL). The presence of both methyl group and fluoro group at the *para* position of the phenyl rings at C-4 and C-6 carbons of pyrimidine in compound **41** exerted better activity at a very minimum inhibitory concentration of 12.5 μg/mL against *M. gypsuem*. In addition compound **42** was found active against *Mucor* at an MIC of 6.25 μg/mL while compound **39** was moderatively active on *Rhizopus* (MIC = 12.5 μg/mL). When the two electron donating substitutents ($-CH_3$ and $-OCH_3$) on the aromatic rings of compound **39** were replaced by the electron withdrawing fluorines as in compound **40** the MIC against Rhizopus improved to 6.25 μg/mL. Compounds **36** and **38** with only one fluorine substitutents on the phenyl rings did not show notable activity either against *Mucor* and *Rhizopus*.

3. Conclusion

A four-step synthetic route furnished the target compounds 2-morpholino-*N*-(4,6-diarylpyrimidin-2-yl)acetamides **34**–**42** in good yields and are characterized by their physical and analytical data. The microbiological screening studies carried out to evaluate the antibacterial and antifungal potencies of the newly synthesized 2-morpholino-*N*-(4,6-diarylpyrimidin-2-yl)acetamides **34**–**42** were clearly known from Table 3. A close inspection of the *in vitro* antibacterial and antifungal activity profile in differently electron donating (CH_3 and OCH_3) and electron withdrawing –F functional group substituted phenyl rings of novel target compounds exerted strong antibacterial activity against all the tested bacterial strains. Compounds **36** and **38** against *β-H. streptococcus* and *K. pneumonia*, **40** against *E. coli* and

Table 3
In vitro antibacterial and antifungal activities of 2-morpholino-*N*-(4,6-diarylpyrimidin-2-yl)acetamides **34**–**42** by two-fold serial dilution method.

Microorganisms	Minimum inhibitory concentration (MIC) in μg/mL										
	34	**35**	**36**	**37**	**38**	**39**	**40**	**41**	**42**	Ciprofloxacin	Fluconazole
Staphylococcus aureus	200	50	100	50	200	100	100	12.5	200	25	–
β-Heamolytic streptococcus	100	100	6.25	100	6.25	200	12.5	100	100	50	–
Vibreo cholerae	200	100	100	100	50	12.5	50	12.5	100	50	–
Escherichia coli	200	50	200	200	50	200	6.25	50	50	25	–
Klebsiella pneumonia	50	100	6.25	100	6.25	50	50	50	100	50	–
Pseudomonas	100	50	100	50	100	50	6.25	100	6.25	25	–
Aspergillus flavus	200	100	6.25	100	6.25	100	12.5	200	6.25	–	50
Mucor	100	50	200	200	50	50	6.25	100	6.25	–	25
Rhizopus	100	50	200	100	100	12.5	6.25	50	200	–	25
Microsporum gypsuem	200	50	100	100	200	50	100	12.5	100	–	25

Pseudomonas, showed excellent antibacterial activity by inhibiting the growth of the respective organisms at a minimum inhibitory concentration of 6.25 μg/mL. Moreover, compound **39** and **41** were active against *V. cholerae* at 12.5 μg/mL MIC. Compound **41** was active also on *S. aureus* (MIC = 12.5 μg/mL) and **42** against *Pseudomonas* (MIC = 6.25 μg/mL). Noteworthy compounds **36**, **38** and **42** against *A. flavus* (MIC value = 6.25 μg/mL), compound **41** (12.5 μg/mL) against *M. gypsuem*, **42** against *Mucor* (MIC value = 6.25 μg/mL), compounds **39** and **40** against *Rhizopus* show inhibition at an MIC of 12.5 and 6.25 μg/mL respectively. Though organofluorine compounds were virtually absent as natural products, it was interesting to note that around 25% of drugs in the pharmaceutical pipeline contain at least one fluorine atom. Among the all tested compounds, fluorine substituted compounds **36**, **38**, **40**, **41** and **42** exerted potent antimicrobial activity with fluorine substitution is commonly used in contemporary medicinal chemistry to improve metabolic stability, bioavailability and protein–ligand interactions [24]. The methods of action of these compounds were unknown. These observations may promote a further development of our research in this field. Further development of this group of 2-morpholino-*N*-(4,6-diarylpyrimidin-2-yl)acetamides may lead to compounds with better pharmacological profile than standard antibacterial and antifungal drugs.

4. Experimental

4.1. Chemistry

Performing TLC assessed the reactions and the purity of the products. All the reported melting points were taken in open capillaries and were uncorrected. IR spectra were recorded in KBr (pellet forms) on a Thermo Nicolet-Avatar-330 FT-IR spectrophotometer and noteworthy absorption values (cm^{-1}) alone were listed. ^1H and ^{13}C NMR spectra were recorded at 400 MHz and 100 MHz respectively on Bruker Avance II 400 NMR spectrometer using DMSO-*d* as solvent. The ESI +ve MS spectra were recorded on a Bruker Daltonics LC–MS spectrometer. Satisfactory microanalysis was obtained on Carlo Erba 1106 CHN analyzer.

By adopting the literature precedent 1,3-diaryl-prop-2-en-1-ones **7**–**15** [25], 2-amino-4,6-diarylpyrimidines **16**–**24** [26] and 2-chloro-*N*-(4,6-diarylpyrimidin-2-yl)acetamides **25**–**33** [27] were synthesized.

4.1.1. General method for the synthesis of 2-morpholino-N-(4,6-diarylpyrimidin-2-yl)acetamides *34*–*42*

A mixture of 2-chloro-*N*-(4,6-diarylpyrimidin-2-yl)acetamides **25**–**33** (0.005 mol), anhydrous potassium carbonate (0.01 mol) and morpholine (0.005 mol) in dry toluene was refluxed for about 8–10 h. After completion of the reaction, potassium carbonate was removed by filtration and excess of solvent was removed under reduced pressure. The obtained residues were purified by column chromatography using benzene and ethylacetate (1:1) mixture as eluent which afforded 2-morpholino-*N*-(4,6-diarylpyrimidin-2-yl)acetamides **34**–**42** in good yields.

4.1.2. 2-Morpholino-N-(4,6-diphenylpyrimidin-2-yl)acetamide *34*

IR (KBr) (cm^{-1}): 3314, 3193, 3058, 3030, 2920, 2851, 1682, 1600, 1565, 1360, 1236, 1112, 761, 693; ^1H NMR (δ ppm): 2.65–2.63 (t, 4H, $N(CH_2)_2$, $J = 4.5$ Hz), 3.49–3.47 (t, 4H, $O(CH_2)_2$, $J = 4.8$ Hz), 3.91 (s, 2H, CH_2), 6.69 (s, 1H, H-5), 8.20–7.30 (m, 10H, H_{arom}), 10.25 (bs, 1H, NH); ^{13}C NMR (δ ppm): 45.89 $N(CH_2)_2$, 66.23 CH_2, 67.25 $O(CH_2)_2$, 101.27 C-5, 126.87–130.30 $-C_{arom}$, 134.55, 137.38 *ipso* carbons, 163.91 C-2, 164.72 C-4, 164.72 C-6, 169.40 C=O.

4.1.3. 2-Morpholino-N-(4-(4-methylphenyl)-6-phenylpyrimidin-2-yl)acetamide *35*

IR (KBr) (cm^{-1}): 3325, 3196, 3058, 3032, 2952, 2920, 2852, 1683, 1611, 1536, 1360, 1233, 1113, 1018, 769, 694; ^1H NMR (δ ppm): 2.37 (s, 3H, CH_3 of phenyl ring), 2.68–2.66 (t, 4H, $N(CH_2)_2$, $J = 4.8$ Hz), 3.48–3.46 (t, 4H, $O(CH_2)_2$, $J = 4.4$ Hz), 3.89 (s, 2H, CH_2), 6.67 (s, 1H, H-5), 8.35–7.25 (m, 9H, H_{arom}), 10.26 (bs, 1H, NH); ^{13}C NMR (δ ppm): 20.89 CH_3, 45.99 $N(CH_2)_2$, 66.23 CH_2, 67.27 $O(CH_2)_2$, 101.45 C-5, 126.88–130.30 $-C_{arom}$, 131.11, 134.52, 137.39, 140.17 *ipso* carbons, 163.95 C-2, 164.70 C-4, 164.70 C-6, 169.45 C=O.

4.1.4. N-(4(4-Fluorophenyl)-6-phenylpyrimidin-2-yl)2-morpholinoacetamide *36*

IR (KBr) (cm^{-1}): 3366, 3207, 3056, 2953, 2917, 2851, 1670, 1600, 1540, 1361, 1229, 1113, 1019, 770, 694; ^1H NMR (δ ppm): 2.65–2.63 (t, 4H, $N(CH_2)_2$, $J = 4.5$ Hz), 3.49–3.46 (t, 4H, $O(CH_2)_2$, $J = 4.7$ Hz), 3.98 (s, 2H, CH_2), 6.72 (s, 1H, H-5), 8.45–7.02 (m, 9H, H_{arom}), 10.30 (bs, 1H, NH); ^{13}C NMR (δ ppm): 45.99 $N(CH_2)_2$, 66.24 CH_2, 67.28 $O(CH_2)_2$, 101.59 C-5, 115.33–131.21 $-C_{arom}$, 133.79, 136.14, 137.27 *ipso* carbons, 162.37 C-2, 163.72 C-6, 163.94 C-4, 168.30 C=O.

4.1.5. N-(4-Phenyl-6-(4-methoxyphenyl)-pyrimidin-2-yl)2-morpholinoacetamide *37*

IR (KBr) (cm^{-1}): 3331, 3199, 3060, 2962, 2920, 2850, 1687, 1605, 1570, 1362, 1245, 1113, 831, 771, 693; ^1H NMR (δ ppm): 2.68–2.66 (t, 4H, $N(CH_2)_2$, $J = 4.3$ Hz), 3.51–3.49 (t, 4H, $O(CH_2)_2$, $J = 4.6$ Hz), 3.85 (s, 3H, OCH_3 of phenyl ring), 3.88 (s, 2H, CH_2), 6.65 (s, 1H, H-5), 8.37–7.06 (m, 9H, H_{arom}), 10.24 (bs, 1H, NH); ^{13}C NMR (δ ppm): 46.02 $N(CH_2)_2$, 55.27 $-OCH_3$, 66.24 CH_2, 67.29 $O(CH_2)_2$, 101.01 C-5, 113.89–128.49 $-C_{arom}$, 129.05, 136.87, 137.76, *ipso* carbons, 163.85 C-2, 164.48 C-4, 164.75 C-6, 171.01 C=O.

4.1.6. N-(4-Phenyl-6-(4-fluorophenyl)-pyrimidin-2-yl)2-morpholinoacetamide *38*

IR (KBr) (cm^{-1}): 3370, 3203, 3061, 2953, 2920, 2852, 1682, 1600, 1542, 1360, 1225, 1113, 1016, 767, 694; ^1H NMR (δ ppm): 2.66–2.64 (t, 4H, $N(CH_2)_2$, $J = 4.4$ Hz), 3.49–3.47 (t, 4H, $O(CH_2)_2$, $J = 4.6$ Hz), 3.93 (s, 2H, CH_2), 6.72 (s, 1H, H-5), 7.97–7.04 (m, 9H, H_{arom}), 10.30 (bs, 1H, NH); ^{13}C NMR (δ ppm): 45.87 $N(CH_2)_2$, 66.07 CH_2, 67.13 $O(CH_2)_2$, 101.59 C-5, 114.71–131.21 $-C_{arom}$, 133.07, 136.55, 137.28 *ipso* carbons, 161.84 C-2, 163.94 C-4, 163.97 C-6, 171.22 C=O.

4.1.7. N-(4-(4-Methoxyphenyl)-6-(4-methylphenyl)pyrimidin-2-yl)2-morpholinoacetamide *39*

IR (KBr) (cm^{-1}): 3373, 3199, 2956, 2920, 2851, 1676, 1607, 1535, 1362, 1300, 1112, 818, 728, 668; ^1H NMR (δ ppm): 2.35 (s, 3H, CH_3 of phenyl ring), 2.65–2.63 (t, 4H, $N(CH_2)_2$, $J = 4.5$ Hz), 3.49–3.46 (t, 4H, $O(CH_2)_2$, $J = 4.7$ Hz), 3.82 (s, 3H, OCH_3 of phenyl ring), 3.85 (s, 2H, CH_2), 6.58 (s, 1H, H-5), 8.19–7.02 (m, 8H, H_{arom}), 10.18 (bs, 1H, NH); ^{13}C NMR (δ ppm): 20.82 CH_3, 46.02 $N(CH_2)_2$, 55.26 $-OCH_3$, 66.32 CH_2, 67.30 $O(CH_2)_2$, 100.66 C-5, 113.87–129.11 $-C_{arom}$, 129.67, 134.66, 140.02 *ipso* carbons, 161.15 C-2, 163.84 C-4, 164.24 C-6, 171.27 C=O.

4.1.8. N-(4,6-Bis(4-fluorophenyl)pyrimidin-2-yl)2-morpholinoacetamide *40*

IR (KBr) (cm^{-1}): 3395, 3215, 3068, 2958, 2919, 2851, 1674, 1601, 1541, 1363, 1228, 1113, 833, 669, 566; ^1H NMR (δ ppm): 2.66–2.64 (t, 4H, $N(CH_2)_2$, $J = 4.9$ Hz), 3.49–3.47 (t, 4H, $O(CH_2)_2$, $J = 4.6$ Hz), 3.97 (s, 2H, CH_2), 6.62 (s, 1H, H-5), 8.45–6.74 (m, 8H, H_{arom}), 10.30 (bs, 1H, NH); ^{13}C NMR (δ ppm): 45.93 $N(CH_2)_2$, 66.07 CH_2, 67.20 $O(CH_2)_2$, 101.37 C-5, 114.29–130.20 $-C_{arom}$, 133.73, 131.47 *ipso* carbons, 162.42 C-2, 163.80 C-6, 163.80 C-4, 170.37 C=O.

4.1.9. N-(4-(4-Methylphenyl)-6-(4-fluorophenyl)pyrimidin-2-yl)2-morpholinoacetamide ***41***

IR (KBr) (cm^{-1}): 3340, 3200, 3062, 3032, 2952, 2920, 2852, 1680, 1603, 1537, 1361, 1226, 1113, 1016, 815, 724, 689; ^{1}H NMR (δ ppm): 2.36 (s, 3H, CH_3), 2.66–2.64 (t, 4H, $N(CH_2)_2$, $J = 4.3$ Hz), 3.49–3.47 (t, 4H, $O(CH_2)_2$, $J = 4.6$ Hz), 3.95 (s, 2H, CH_2), 6.68 (s, 1H, H-5), 8.28–7.27 (m, 8H, H_{arom}), 10.26 (bs, 1H, NH); ^{13}C NMR (δ ppm): 20.90 –CH_3, 45.93 $N(CH_2)_2$, 66.08 CH_2, 67.21 $O(CH_2)_2$, 101.24 C-5, 115.31–127.98 –C_{arom}, 129.67, 134.66, 140.02 *ipso* carbons, 161.38 C-2, 163.89 C-4, 164.83 C-6, 170.21 C═O.

4.1.10. N-(4-(4-Fluorophenyl)-6-(4-methoxyphenyl)pyrimidin-2-yl)2-morpholinoacetamide ***42***

IR (KBr) (cm^{-1}): 3338, 3207, 3080, 2953, 2917, 2852, 1676, 1600, 1531, 1364, 1294, 1111, 1029, 722, 677; ^{1}H NMR (δ ppm): 2.68–2.66 (t, 4H, $N(CH_2)_2$, $J = 4.8$ Hz), 3.51–3.49 (t, 4H, $O(CH_2)_2$, $J = 4.6$ Hz), 3.84 (s, 3H, OCH_3), 3.87 (s, 2H, CH_2), 6.66 (s, 1H, H-5), 8.46–7.05 (m, 8H, H_{arom}), 10.25 (bs, 1H, NH),; ^{13}C NMR (δ ppm): 46.01 $N(CH_2)_2$, 55.27 –OCH_3, 66.25 CH_2, 67.31 $O(CH_2)_2$, 100.82 C-5, 113.89–129.07 –C_{arom}, 129.27, 129.56, 129.79, 133.93, *ipso* carbons, 161.25 C-2, 162.31 C-6, 163.65 C-4, 168.55 C═O.

4.2. Microbiology

4.2.1. Materials

All the clinically isolated bacterial strains namely *S. aureus*, *β-H. streptococcus*, *Vibreo cholerae*, *E. coli*, *K. pneumonia*, *P. aeruginosa* and fungal strains namely *A. flavus*, *Mucor*, *Rhizopus* and *M. gypsuem* were obtained from Faculty of Medicine, Annamalai University, Annamalainagar 608 002, Tamil Nadu, India.

4.2.2. In vitro antibacterial and antifungal activity

MIC in μg/mL values was carried out by two-fold serial dilution method [28]. The respective test compounds **34**–**42** were dissolved in dimethyl sulphoxide (DMSO) to obtain 1 mg mL^{-1} stock solution. Seeded broth (broth containing microbial spores) was prepared in NB from 24 h old bacterial cultures on nutrient agar (Hi-media, Mumbai) at 37 ± 1 °C while fungal spores from 1 to 7 days old Sabouraud's agar (Hi-media, Mumbai) slant cultures were suspended in SDB. The colony forming units (cfu) of the seeded broth were determined by plating technique and adjusted in the range of 10^4–10^5 cfu/mL. The final inoculums size was 10^5 cfu/mL for antibacterial assay and $1.1–1.5 \times 10^2$ cfu/mL for antifungal assay. Testing was performed at pH 7.4 ± 0.2 for bacteria (NB) and at a pH 5.6 for fungi (SDB). Exactly 0.4 mL of the solution of test compound was added to 1.6 mL of seeded broth to form the first dilution. One milliliter of this was diluted with a further 1 mL of seeded broth to give the second dilution and so on till six such dilutions were obtained. A set of assay tubes containing only seeded broth was kept as control. The tubes were incubated in BOD incubators at 37 ± 1 °C for bacteria and 28 ± 1 °C for fungi. MICs were recorded by visual observations after 24 h (for bacteria) and 72–96 h (for fungi) of incubation. Ciprofloxacin was used as standard for bacteria studies and Fluconazole was used as standards for fungal studies.

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