친환경적 One Pot 합성, *In Vitro*에서 Morpholino Pyrimidines의 분광학적 특성과 항균성 및 항진균성

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A Green Chemical Approach towards the 'One-Pot' Synthesis, Spectral Characterization and *in Vitro* Antibacterial and Antifungal Activities of Morpholino Pyrimidines

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요약. NaHSO₄.SiO₂ 촉매를 이용한 microwave 및 무수조건하에서 새로운 morpholino pyrimidines의 One-pot합성이다. 합성된 모든 화합물을 이용하여 *in vitro*에서 임상학적인 단리된 박테리아 품종 즉 Bacillus subtilis, Bacillus cerues, Micrococcus luteus , Salmonella typhii에 대해 항균성을 살펴보았고, 균품종 즉 Aspergillus niger, Candida 6, Candida 51에 대해 항진균성을 살펴보았다. 미생물 결과에 대한 합성된 화합물의 구조적 활동성의 관련성에 대해 토론하였다.

주제어: 'One-pot' 합성, (E)-1-(4-morpholinophenyl)-3-aryl-prop-2-en-1-ones, Morpholino pyrimidines, 항균성, 항진균성

ABSTRACT. 'One-pot' reaction procedure for the synthesis of novel morpholino pyrimidines (**10-18**) under microwave irradiation in 'dry media' in the presence of heterogeneous NaHSO₄.SiO₂ catalyst was developed. All the synthesized compounds were screened for their *in vitro* antibacterial activities against clinically isolated bacterial strains namely *Bacillus subtilis, Bacillus cerues, Micrococcus luteus* and *Salmonella typhii* and antifungal activities against fungal strains namely *Aspergillus niger, Candida 6* and *Candida 51*. Structure activity relationship of the synthesized compounds against microbiological results was discussed.

Keywords: 'One-pot' synthesis, (E)-1-(4-morpholinophenyl)-3-aryl-prop-2-en-1-ones, Morpholino pyrimidines, Antibacterial activities, Antifungal activities

INTRODUCTION

Organic heterocycles are of considerable importance from both the chemical and biological viewpoints. Synthesis of molecules that are novel but still resemble known biologically active molecules by virtue of the presence of some critical structural features is an essential component of the search for new leads in drug design programs.[1] A widely used and preferred tool for accelerating organic reactions, microwave irradiation (MWI), has been applied to organic reactions in the absence of solvent and/or in the presence of a solid support, such as clays, alumina and silica, resulting in shorter reaction times

and higher product yields than those obtained by using conventional heating. By using microwave heating it is often possible to enhance the rate of reactions and improve product yields.[2-4] Much of the early microwave-promoted synthesis was performed using domestic (household) microwave apparatus. As well as being inherently unsafe due to the fact that a domestic microwave apparatus is not designed for synthetic chemistry, it can be hard to measure temperature accurately and there can be issues with reproducibility. With the advent of scientific microwave apparatus many of these problems have been overcome.[5] In chemistry a one-pot synthesis is a strategy to improve the efficiency of a chemical reaction whereby a reactant is subjected to successive chemical reactions in just one reactor. This is much desired by chemists because avoiding a lengthy separation process and purification of the intermediate chemical compounds would save time and resources while increasing chemical yield.

Pyrimidines being an integral part of nucleic acids and many chemotherapeutic agents display a wide range of pharmacological activities as bactericide,[6] fungicide,[7] phosphodiesterase inhibitor,[8] viricide,[9] and leishmancide.[10] Pyrimidines are the basic nucleus in nucleic acids and have been associated with a number of biological activities.[11] 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.[12] Many pyrimidine derivatives have been found to be active against different forms of cancer.[13] Various method of synthesis and reactions of aminopyrimidines are reported.[14-16]

Morpholine is a simple heterocyclic compound with a great industrial importance. Many N-fuctionalized morpholines have found to posses diverse pharmacological activites. They are reported to exert a number of important physiological activities such as antidiabetic,[17] antihyperlipo-proteinemics,[18] antiemetic,[19] platelet aggregation inhibitors, bronchodilators and growth stimulats[20] and antidepressants.[21] These were also used in the treatment of inflammatory diseases, pain, migraine and asthma.[22]

The development of efficient syntheses of bioactive compounds such as natural products and analogs, drugs, diagnostics, and agrochemicals in academia and industry is a very important issue of modern chemistry.[23] Modern syntheses must deal carefully with our resources and our time, must reduce the amount of waste formed, should use catalytic transformations, and, finally, must avoid all toxic reagents and solvents. In addition, synthetic methodology must be designed in a way that allows access to diversified substance libraries in an automatized way. A general way to improve synthetic efficiency and also to give access to a multitude of diversified molecules is the development of domino reactions which allow the formation of complex compounds starting from simple substrates in a single transformation consisting of several steps.

Silica gel supported sodium hydrogen sulfate ($NaHSO_4.SiO_2$), a non-toxic and inexpensive catalyst, has been used for one-pot conversion of ketones to amides,[24] synthesis of imines,[25] one-pot synthesis of 1,2,3-selenadiazoles,[26] 1,2,4-triazolidin-3-thiones[27] and Knoevenagel condensation reactions.[28] Owing to our interest in synthesizing fascinating pharmacological and therapeutic important compounds under microwave assisted reactions,[24-28] we attempt and succeed now to use silica gel supported sodium hydrogen sulphate ($NaHSO_4.SiO_2$), as a heterogeneous catalyst for the one-pot synthesis of morpholino pyrimidines (**10-18**) in *dry media* under microwave irradiation and their *in vitro* microbiological evaluation is carried out against clinically isolated bacterial and fungal strains.
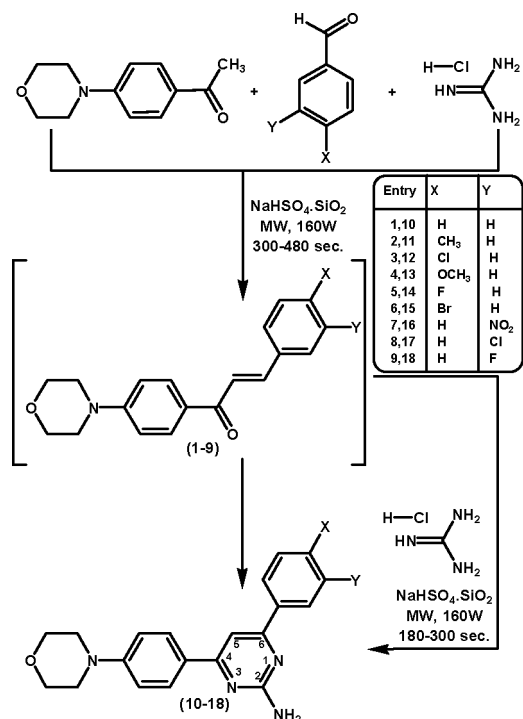
RESULTS AND DISCUSSION

Chemistry

The classical approach for the synthesis of morpholino pyrimidines (**10-18**) is as follows: (*E*)-1-(4-morpholinophenyl)-3-aryl-prop-2-en-1-ones (**1-9**) are synthesized by the condensation of commercially available 1-(4-morpholinophenyl) ethanone and substituted benzaldehyde in the presence of sodium hydroxide in ethanol at 20 °C for 1 h. Treatment of compounds (**1-9**) with guanidine nitrate

in the presence of potassium hydroxide in refluxing ethanol for 12 h yields the respective 4-(4-morpholinophenyl)-6-aryl-pyrimidin-2-amines (**10-18**). There are some problems associated with above synthesis, such as severe conditions, very low yields for the reaction, use of corrosive bases like potassium hydroxide,[29] difficulty in separating the products from the system and longer reaction times. In the present 'one-pot' procedure, treatment of various substituted 0.005 mol of benzaldehyde with 0.005 mol of 1-(4-morpholinophenyl) ethanone and 0.005 mol of guanidine hydrochloride along with a catalytic amount of $NaHSO_4.SiO_2$ (25 mg) afford the corresponding morpholino pyrimidines (**10-18**) (*Scheme* 1 & *Table* 1) in high yields in dry media under MW irradiation at a power level of 160 W. $NaHSO_4.SiO_2$ catalyst was shown to be one of the most efficient MW absorber with a very high specificity to MW heating. It was able to reach a temperature of 110 °C after 4 minutes of irradiation in a domestic oven (160 W). Mere 25 mg of $NaHSO_4$.

SiO_2 catalyst to 0.005 moles of substrates is the most acceptable ratio in terms of efficiency and safety; a power level of 160 watts is the most suitable one.

Formation of morpholino pyrimidines (**10-18**) by this method is believed to be followed *via* the (*E*)-1-(4-morpholinophenyl)-3-aryl-prop-2-en-1-ones (**1-9**). In the first step, three component condensation of 1-(4-morpholinophenyl) ethanone, substituted benzaldehyde and guanidine hydrochloride are converted to their respective intermediate (*E*)-1-(4-morpholinophenyl)-3-aryl-prop-2-en-1-ones (**1-9**) and rapidly rearrange to give morpholino pyrimidines (**10-18**) in the second step. The attempt to isolate the respective intermediate (**1-9**) from the reaction mixture is successful. The formations of morpholino pyrimidines (**10-18**) *via* the intermediate (*E*)-1-(4-morpholinophenyl)-3-aryl-prop-2-en-1-ones (**1-9**) are confirmed by the same kind of reactions carried out using $NaHSO_4.SiO_2$ catalyst and guanidine hydrochloride (**10-18**) and under microwave irradiation. The products formed from the above two methods are found to be the same. The structures of all the synthesized compounds (**1-18**) are discussed with the help of m.p.'s, elemental analysis, FT-IR, MS, one-dimensional NMR (^{1}H, ^{13}C) spectra.

The reaction involved in the formation of morpholino pyrimidines might proceed either by route (i) 1,4-addition or route (ii) 1,2-addition of the guanidine to (*E*)-1-(4-morpholinophenyl)-3-aryl-prop-2-en-1-ones (**1-9**), followed by cyclisation, proton shift and aromatization to afford the morpholino pyrimidines (**10-18**) (*Scheme* 2).

Antibacterial activity

All the synthesized morpholino pyrimidines **10-18** are tested for their antibacterial activity *in vitro* against *B.subtilis, B.cerues, M.luteus* and *S.typhii* and Penicillin is used as standard drug. Minimum inhibitory concentration (MIC) in µg/mL values is reproduced in *Table* 2 and their pictorial representation is shown in *Fig.* 1. Among the synthesized compounds, compound 10 which is having no substitution at the *para* position of phenyl rings attached

Entry	X	Y
1,10	H	H
2,11	CH₃	H
3,12	Cl	H
4,13	OCH₃	H
5,14	F	H
6,15	Br	H
7,16	H	NO₂
8,17	H	Cl
9,18	H	F

Scheme 1. Novel 'one-pot' synthesis of morpholino pyrimidines in '*dry media*'

Table 1. Physical and analytical data of (*E*)-1-4-morpholinophenyl)-3-aryl-prop-2-en-1-ones (**1-9**) and 4-(4-morpholinophenyl)-6-arylpyrimidin-2-amines (**10-18**)

Entry	X	Y	Yield (%)	m.p °C	Elemental analysis (%)			m/z (M+1)⁻ Molecular formula
					C Found (calculated)	H Found (calculated)	N Found (calculated)	
1	H	H	98	149	77.78 (77.81)	6.46 (6.48)	4.76 (4.77)	294 $C_{19}H_{19}NO_2$
2	CH₃	H	92	179	78.15 (78.17)	6.81 (6.84)	4.54 (4.56)	308 $C_{20}H_{21}NO_2$
3	Cl	H	90	143	69.70 (69.72)	5.47 (5.50)	4.26 (4.28)	328 $C_{19}H_{18}NO_2Cl$
4	OCH₃	H	95	111	74.29 (74.30)	6.48 (6.50)	4.31 (4.33)	324 $C_{20}H_{21}NO_3$
5	F	H	95	160	73.29 (73.31)	5.76 (5.78)	4.48 (4.50)	312 $C_{19}H_{18}NO_2F$
6	Br	H	95	145	61.27 (61.30)	4.83 (4.87)	3.74 (3.76)	372 $C_{19}H_{18}BrNO_2$
7	H	NO₂	90	135	67.43 (67.45)	5.29 (5.32)	8.26 (8.28)	339 $C_{19}H_{18}N_2O_4$
8	H	Cl	90	138	69.69 (69.72)	5.48 (5.50)	4.25 (4.28)	328 $C_{19}H_{18}NO_2Cl$
9	H	F	95	154	73.28 (73.31)	5.74 (5.78)	4.47 (4.50)	312 $C_{19}H_{18}NO_2F$
10	H	H	90	118	72.05 (72.09)	6.28 (6.30)	16.77 (16.80)	333 $C_{20}H_{21}N_4O$
11	CH₃	H	95	73	72.61 (72.64)	6.59 (6.62)	16.10 (16.12)	347 $C_{21}H_{23}N_4O$
12	Cl	H	90	123	65.31 (65.33)	5.41 (5.44)	15.20 (15.23)	367 $C_{20}H_{20}N_4OCl$
13	OCH₃	H	95	91	69.41 (69.44)	6.31 (6.33)	15.38 (15.41)	363 $C_{21}H_{23}N_4O_2$
14	F	H	90	87	68.38 (68.40)	5.66 (5.69)	15.91 (15.94)	351 $C_{20}H_{20}N_4OF$
15	Br	H	90	93	58.36 (58.40)	4.64 (4.66)	13.59 (13.62)	411 $C_{20}H_{19}BrN_4O$
16	H	NO₂	80	176	65.92 (65.96)	5.47 (5.49)	15.35 (15.38)	364 $C_{20}H_{20}N_5O_3$
17	H	Cl	85	135	65.30 (65.33)	5.42 (5.44)	15.20 (15.23)	367 $C_{20}H_{20}N_4OCl$
18	H	F	85	96	68.37 (68.40)	5.68 (5.69)	15.92 (15.94)	351 $C_{20}H_{20}N_4OF$

to C-4 and C-6 carbons of pyrimidine moiety exerted moderate activities against *B.subtilis* and *S.typhii* and exerted poor activity against *B.cerues* and *M. luteus*. All the other synthesized compounds **11-18** exhibit a wide range of antibacterial potency against the tested strains except compounds **15** and **16** which did not show any activity against *S.typhii* and *M.*

luteus. Structure-activity relationship results for the synthesized compounds have shown that compound **11** which is having electron donating methyl substitution at the *para* position of phenyl rings attached to C-4 and C-6 carbons of pyrimidine moiety shows excellent antibacterial activity against *B.cerues* and *S.typhii* at a MIC value of 6.25 and 12.5 μg/mL
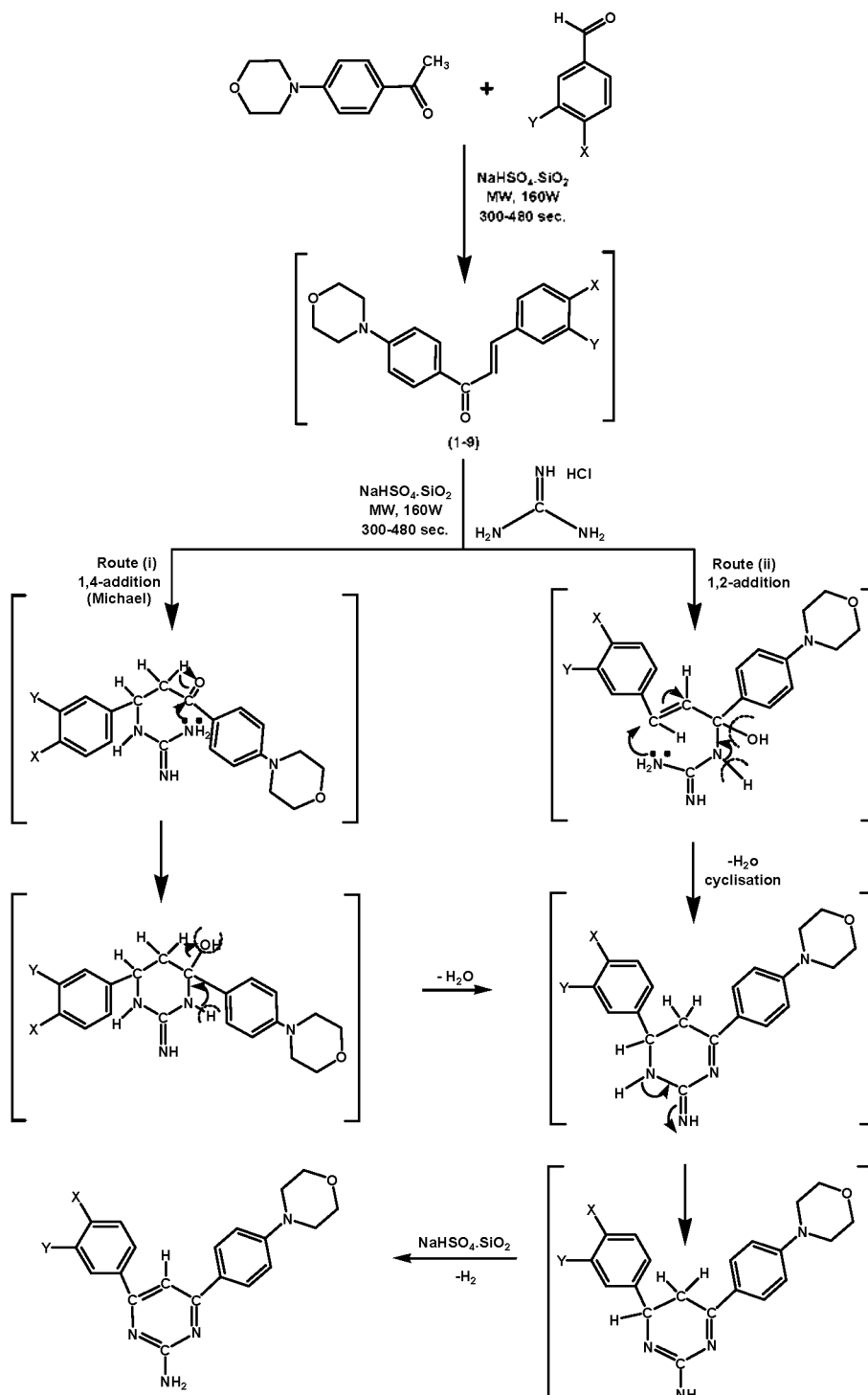
Scheme 2. Reaction mechanism for the formation of morpholino pyrimidines (10-18)

Table 2. *In vitro* antibacterial activities of morpholino pyrimidines

Microorganisms	Minimum Inhibitory Concentration (MIC) in μg/mL									
	10	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**Penicillin**
B.subtilis	25	50	6.25	100	12.5	25	6.25	12.5	25	25
B.cerues	100	6.25	12.5	25	6.25	50	25	12.5	12.5	12.5
M.luteus	200	25	25	25	6.25	6.25	$-^a$	25	6.25	12.5
S.typhii	25	12.5	6.25	12.5	25	$-^a$	6.25	12.5	12.5	25

[a]No inhibition even at higher concentration i.e.. at 200 μg/mL
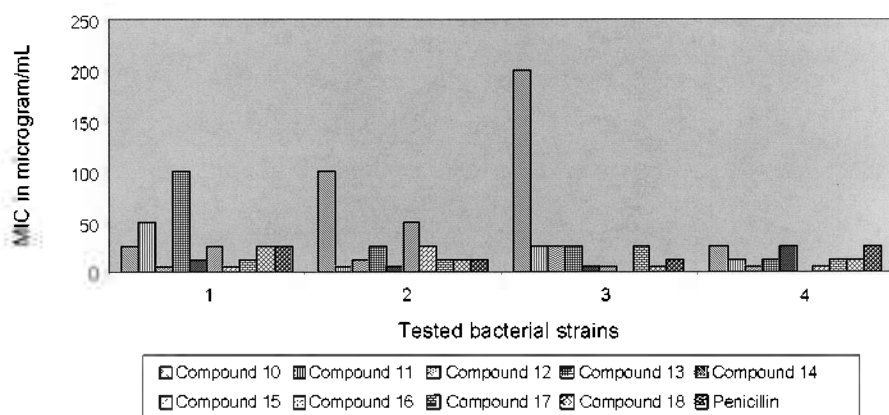
Fig. 1. Pictorial representation of the *in vitro* antibacterial activities of morpholino pyrimidines.

respectively. Similarly chloro substituted compound **17** is active against all the tested strains. Compound **12** which is having electron withdrawing chloro substitution at the *para* position of phenyl rings attached to C-4 and C-6 carbons of pyrimidine moiety shows excellent antibacterial activity against *B.subtilis* and *S.typhii* at a MIC value of 6.25 μg/mL. Methoxy substituted compound **13** exerted potent activity against *S.typhii*. Fluoro substituted compound **14** and **18** exerted excellent activity against *B.cerues* and *M.luteus*. Compound **15** which is having electron withdrawing bromo substitution at the *para* position of phenyl rings attached to C-4 and C-6 carbons of pyrimidine moiety shows excellent antibacterial activity against *M.luteus*. Compound **16** which is having electron withdrawing nitro substitution at the *para* position of phenyl rings attached to C-4 and C-6 carbons of pyrimidine moiety shows excellent antibacterial activity against all the tested strains except *M.luteus*.
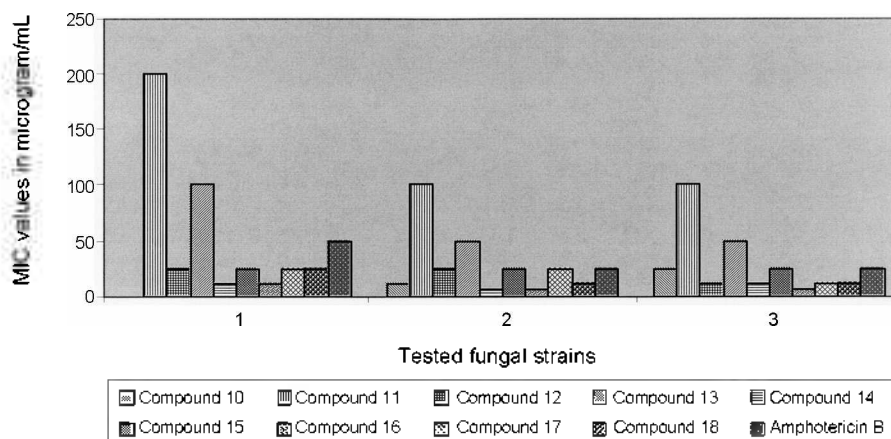
Antifungal activity

The *in vitro* antifungal activity of compounds **10-18** is studied against the fungal strains *viz.*, *Aspergillus niger*, *Candida 6* and *Candida 51*. Amphotericin B is used as a standard drug. Minimum inhibitory concentration (MIC) in μg/mL values is reproduced in *Table* 3 and their pictorial representation is shown in *Fig.* 2 Compound **10** which is having no substitution at the *para* position of phenyl rings attached to C-4 and C-6 carbons of pyrimidine moiety exerted moderate activities against *Candida 51* and exerted good activity against *Candida 6* but did not show any activity against *A.niger* even at a higher concentration of 200 μg/mL. Compounds **11** and **13** which is having electron donating methyl/methoxy substitution at the *para* position of phenyl rings attached to C-4 and C-6 carbons of pyrimidine moiety respectively and bromo substituted compound **15** exerted moderate activity against all the tested fungal strains. Compounds **12** and **17** which

Table 3. *In vitro* antifungal activities of morpholino pyrimidines

Microorganisms	Minimum Inhibitory Concentration (MIC) in μg/mL									
	10	11	12	13	14	15	16	17	18	Amphotericin B
A.niger	-[a]	200	25	100	12.5	25	12.5	25	25	50
Candida 6	12.5	100	25	50	6.25	25	6.25	25	12.5	25
Candida 51	25	100	12.5	50	12.5	25	6.25	12.5	12.5	25

[a]No inhibition even at higher concentration i.e., at 200 μg/mL

Fig. 2. Pictorial representation of the *in vitro* antifungal activities of morpholino pyrimidines.

is having electron withdrawing chloro substitution at the *para/meta* position of phenyl rings attached to C-4 and C-6 carbons of pyrimidine moiety respectively exerted good activity against all the tested fungal strains. Compounds **14** and **18** which is having electron withdrawing fluoro substitution at the *para/meta* position of phenyl rings attached to C-4 and C-6 carbons of pyrimidine moiety respectively exerted excellent activity against all the tested fungal strains. Nitro substituted compound **16** exerted very good antifungal activity against all the tested fungal strains namely *Aspergillus niger, Candida 6* and *Candida 51* at a MIC value of 12.5, 6.25 and 6.25 μg/mL respectively.

CONCLUSION

In conclusion, we have developed an efficient, environmentally friendly, one-pot microwave-assisted synthesis of a new series of novel morpholino pyrimidines in good to excellent yields. The method

not only offers substantial increase in the yield over conventional method but also eliminates the usage of solvents like ethanol/methanol, corrosive bases like KOH and shortens the reaction time from several hours of reflux to few seconds.[29] The microbiological screening studies carried out to evaluate the antibacterial and antifungal potencies of the newly synthesized morpholino pyrimidines **10-18** are clearly known from *Table* 2 and *Table* 3. Antimicrobial results of the synthesized compounds against the tested bacterial and fungal strains shows that compounds **12** and **14-18,** which have electron withdrawing chloro, fluoro, bromo and nitro functional groups at the *para/meta* position of phenyl rings attached to C-4 and C-6 carbons of pyrimidine moiety delivered excellent antibacterial and antifungal activities. These observations may promote a further development of our research in this field. Further development of this group of morpholino pyrimidines may lead to compounds with better pharmacological profile than standard antibacterial

and antifungal drugs.

EXPERIMENTAL

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 note worthy absorption values (cm^{-1}) alone are listed. ^{1}H and ^{13}C NMR spectra were recorded at 400 MHz and 100 MHz respectively on Bruker AMX 400 NMR spectrometer using DMSO-d as solvent. The electron spray impact (ESI) positive (+ve) mass (MS) spectra were recorded on a Bruker Daltonics LC-MS spectrometer. Satisfactory microanalysis was obtained on Carlo Erba 1106 CHN analyzer. BIOTAGE Initiator microwave synthesizer. Sweden a scientific microwave oven was used for the irradiation.

General method for the 'one-pot' microwave assisted synthesis of morpholino pyrimidines catalyzed by NaHSO$_4$.SiO$_2$ (10-18)

A mixture of 1-(4-morpholinophenyl) ethanone (0.005 mol), substituted benzaldehyde (0.005 mol), guanidine hydrochloride (0.005 mol), and NaHSO$_4$.SiO$_2$ (25 mg) was added in an alumina bath and mixed properly with the aid of glass rod (10 sec.) and then irradiated in a microwave oven for 300 - 480 sec. at 320 W (monitored by TLC). After completion of the reaction, the reaction mixture was extracted with ethyl acetate (3 × 5 mL). The catalyst and other solid wastes were removed by filtration. The combined organic layer was washed with water three times and then dried over anhydrous MgSO$_4$. The organic layer was concentrated *in vacuo* to furnish the products (10-18), which were purified by recrystallization in ethanol.

The reaction is also carried out from the intermediate (E)-1-(4-morpholinophenyl)-3-aryl-prop-2-en-1-ones (1-9), guanidine hydrochloride and NaHSO$_4$.SiO$_2$ (25 mg) was added in an alumina bath and mixed properly with the aid of glass rod (10 sec.) and then irradiated in a microwave oven

for 180 - 300 sec. at 160 W (monitored by TLC). After completion of the reaction, the reaction mixture was extracted with ethyl acetate (3 × 5 mL). The catalyst and other solid wastes were removed by filtration. The combined organic layer was washed with water three times and then dried over anhydrous MgSO$_4$. The organic layer was concentrated *in vacuo* to furnish the products (10-18), which were purified by recrystallization in ethanol.

(E)-1-(4-morpholinophenyl)-3-phenyl-prop-2-en-1-one (1)

IR (KBr) ν (cm^{-1}): 3007, 2962, 2924, 2852, 1646, 1606, 1190, 769; ^{1}H NMR (δ ppm): 3.33-3.36 (t, 4H, N(CH$_2$)$_2$, J = 4.7 Hz), 3.87-3.89 (t, 4H, O(CH$_2$)$_2$, J = 4.7 Hz), 6.93-6.95 (d, 1H, H$_2$, J = 8.9 Hz); 7.38-7.82 (m, 10H, H$_{arom}$); 8.01-8.03 (d, 1H, H$_3$, J = 8.9 Hz); ^{13}C NMR (δ ppm): 47.7 N(CH$_2$), 66.5 O(CH$_2$), 122.3 C-2, 143.2 C-3, 113.6, 128.8-130.3 −C$_{arom}$, 128.2, 135.4, 154.1 *ipso*-C, 188.1 C-1.

(E)-1-(4-morpholinophenyl)-3-p-tolyl-prop-2-en-1-one (2)

IR (KBr) ν (cm^{-1}): 3012, 2923, 2924, 2851, 1645, 1600, 1194, 810; ^{1}H NMR (δ ppm): 1.57 (s, 3H, CH$_3$ at phenyl ring), 3.32-3.35 (t, 4H, N(CH$_2$)$_2$, J = 4.8 Hz), 3.86-3.89 (t, 4H, O(CH$_2$)$_2$, J = 4.8 Hz), 6.92-6.94 (d, 1H, H$_2$, J = 8.8 Hz); 7.21-7.80 (m, 9H, H$_{arom}$); 8.00-8.02 (d, 1H, H$_3$, J = 8.8 Hz); ^{13}C NMR (δ ppm): 21.0 CH$_3$ at phenyl ring, 47.7 N(CH$_2$), 66.5 O(CH$_2$), 121.2 C-2, 143.3 C-3, 113.5, 129.3-130.5 −C$_{arom}$, 128.2,132.7,140.5,154.1 *ipso*-C, 188.2 C-1.

(E)-3-(4-chlorophenyl)-1-(4-morpholinophenyl)-prop-2-en-1-one (3)

IR (KBr) ν (cm^{-1}): 3087, 2967, 2920, 2859, 1597, 1654, 1202, 817; ^{1}H NMR (δ ppm): 3.34-3.37 (t, 4H, N(CH$_2$)$_2$, J = 4.7 Hz), 3.89-3.91 (t, 4H, O(CH$_2$)$_2$, J = 4.8 Hz), 6.97-6.99 (d, 1H, H$_2$, J = 8.8 Hz); 7.35-7.76 (m, 9H, H$_{arom}$); 8.00-8.02 (d, 1H, H$_3$, J = 8.9 Hz); ^{13}C NMR (δ ppm): 47.6 N(CH$_2$), 66.5 O(CH$_2$), 121.2 C-2, 141.8 C-3, 113.5, 129.4-130.6 −C$_{arom}$, 129.1,133.8,135.0,154.0 *ipso*-C, 192.2 C-1.

(E)-3-(4-methoxyphenyl)-1-(4-morpholinophenyl)prop-2-en-1-one (4)

IR (KBr) ν (cm^{-1}): 3010, 2961, 2918, 2841, 1645, 1601, 1225; ^{1}H NMR (δ ppm): 3.32-3.35 (t, 4H, N(CH$_2$)$_2$, J = 4.8 Hz), 3.87-3.90 (t, 4H, O(CH$_2$)$_2$, J =

4.8 Hz), 3.86 (s, 3H, OCH$_3$ at phenyl ring), 7.59-7.61 (d, 1H, H$_2$, J = 8.6 Hz); 6.92-7.46 & 7.75-7.79 (m, 9H, H$_{arom}$); 8.00-8.02 (d, 1H, H$_3$, J = 8.7 Hz); ^{13}C NMR (δ ppm): 47.6 N(CH$_2$), 55.3 OCH$_3$ at phenyl ring, 66.5 O(CH$_2$), 119.6 C-2, 143.1 C-3, 113.5, 129.2-130.4 −C$_{arom}$, 127.9,129.9, 153.9, 161.3 *ipso*-C, 187.8 C-1.

(E)-3-(4-fluorophenyl)-1-(4-morpholinophenyl)-prop-2-en-1-one (5)

IR (KBr) v (cm^{-1}): 3009, 2969, 2919, 2849, 1650, 1602, 1227; ^{1}H NMR (δ ppm): 3.33-3.36 (t, 4H, N(CH$_2$)$_2$, J = 4.7 Hz), 3.87-3.89 (t, 4H, O(CH$_2$)$_2$, J = 4.8 Hz), 6.93-6.95 (d, 1H, H$_2$, J = 8.9 Hz); 7.08-7.78 (m, 9H, H$_{arom}$); 8.00-8.02 (d, 1H, H$_3$, J = 8.9 Hz); ^{13}C NMR (δ ppm): 47.5 N(CH$_2$), 66.5 O(CH$_2$), 121.6 C-2, 141.9 C-3, 113.4, 115.8, 130.1,131.5 −C$_{arom}$, 128.8, 130.6, 154.1, 162.5 *ipso*-C, 187.8 C-1.

(E)-3-(4-bromophenyl)-1-(4-morpholinophenyl)-prop-2-en-1-one (6)

IR (KBr) v (cm^{-1}): 3001, 2960, 2923, 2845, 1657, 1612, 1227; ^{1}H NMR (δ ppm): 3.32-3.35 (t, 4H, N(CH$_2$)$_2$, J = 4.5 Hz), 3.86-3.87 (t, 4H, O(CH$_2$)$_2$, J = 4.6 Hz), 6.94-6.96 (d, 1H, H$_2$, J = 8.8 Hz); 7.18-7.82 (m, 9H, H$_{arom}$); 8.01-8.03 (d, 1H, H$_3$, J = 8.6 Hz); ^{13}C NMR (δ ppm): 47.9 N(CH$_2$), 65.6 O(CH$_2$), 121.8 C-2, 142.3 C-3, 113.8, 115.1, 130.7, 131.7 −C$_{arom}$, 128.5, 131.2, 154.7, 162.7 *ipso*-C, 188.8 C-1.

(E)-1-(4-morpholinophenyl)-3-(3-nitrophenyl)-prop-2-en-1-one (7)

IR (KBr) v (cm^{-1}): 3087, 2966, 2923, 2862, 1651, 1608, 1224; ^{1}H NMR (δ ppm): 3.36-3.38 (t, 4H, N(CH$_2$)$_2$, J = 4.5 Hz), 3.88-3.90 (t, 4H, O(CH$_2$)$_2$, J = 4.6 Hz), 6.95-6.97 (d, 1H, H$_2$, J = 8.9 Hz); 7.27-7.91 & 8.23-8.25 (m, 9H, H$_{arom}$); 8.03-8.05 (d, 1H, H$_3$, J = 8.9 Hz); ^{13}C NMR (δ ppm): 47.3 N(CH$_2$), 66.9 O(CH$_2$), 122.0 C-2, 140.1 C-3, 113.3, 124.2-134.2 −C$_{arom}$, 128.9, 137.1, 148.7, 154.3 *ipso*-C, 187.8 C-1.

(E)-1-(4-morpholinophenyl)-3-(3-chlorophenyl)-prop-2-en-1-one (8)

IR (KBr) v (cm^{-1}): 3093, 2969, 2928, 2857, 1593, 1652, 1212, 830; ^{1}H NMR (δ ppm): 3.33-3.36 (t, 4H, N(CH$_2$)$_2$, J = 4.6 Hz), 3.89-3.91 (t, 4H, O(CH$_2$)$_2$, J = 4.6 Hz), 6.96-6.98 (d, 1H, H$_2$, J = 8.9 Hz); 7.33-7.81 (m, 9H, H$_{arom}$); 7.98-8.00 (d, 1H, H$_3$, J =

8.7 Hz); ^{13}C NMR (δ ppm): 47.8 N(CH$_2$), 66.4 O(CH$_2$), 121.2 C-2, 141.6 C-3, 113.3, 128.8-130.1 −C$_{arom}$, 129.3, 133.7, 145.2, 154.1 *ipso*-C, 192.3 C-1.

(E)-1-(4-morpholinophenyl)-3-(3-fluorophenyl)-prop-2-en-1-one (9)

IR (KBr) v (cm^{-1}): 3018, 2974, 2924, 2843, 1649, 1605, 1226; ^{1}H NMR (δ ppm): 3.33-3.36 (t, 4H, N(CH$_2$)$_2$, J = 4.8 Hz), 3.86-3.88 (t, 4H, O(CH$_2$)$_2$, J = 4.7 Hz), 6.92-6.94 (d, 1H, H$_2$, J = 8.8 Hz); 7.18-7.68 (m, 9H, H$_{arom}$); 7.92-7.94 (d, 1H, H$_3$, J = 8.7 Hz); ^{13}C NMR (δ ppm): 47.5 N(CH$_2$), 66.6 O(CH$_2$), 121.3 C-2, 141.8 C-3, 113.4, 125.3-130.1 −C$_{arom}$, 128.6, 130.5, 154.3, 162.7 *ipso*-C, 187.5 C-1.

4-(4-morpholinophenyl)-6-phenylpyrimidin-2-amine (10)

IR (KBr) (cm^{-1}): 3355, 3459, 3060, 2961, 2920, 1661, 1599, 1229, 928, 824, 776, 697, 634; ^{1}H NMR (δ ppm): 3.33-3.38 (t, 4H, N(CH$_2$)$_2$, J = 4.7 Hz), 3.88-3.89 (t, 4H, O(CH$_2$)$_2$, J = 4.8 Hz), 5.23 (s, 2H, NH$_2$), 7.38-7.85 (m, 10H, H$_{arom}$), the signal for H-5 proton may be merged with the aromatic protons; ^{13}C NMR (δ ppm): 46.3 N(CH$_2$), 67.3 O(CH$_2$), 103.4 C-5, 163.8 C-2, 164.7 C-6, 165.0 C-4, 127.0-131.5 −C$_{arom}$, 142.1, 153.9, *ipso*-C.

4-(4-morpholinophenyl)-6-p-tolylpyrimidin-2-amine (11)

IR (KBr) (cm^{-1}): 3432, 3200, 2967, 2923, 1625, 1599, 1229, 928, 815, 645; ^{1}H NMR (δ ppm): 2.31 (s, 3H, CH$_3$), 3.34-3.38 (t, 4H, N(CH$_2$)$_2$, J = 4.8 Hz), 3.86-3.89 (t, 4H, O(CH$_2$)$_2$, J = 4.8 Hz), 5.25 (s, 2H, NH$_2$), 7.20-8.12 (m, 9H, H$_{arom}$), the signal for H-5 proton may be merged with the aromatic protons; ^{13}C NMR (δ ppm): 25.4 CH$_3$, 46.7 N(CH$_2$), 67.5 O(CH$_2$), 104.1 C-5, 163.8 C-2, 164.1 C-6, 164.3 C-4, 126.0-131.4 −C$_{arom}$, 143.8, 152.4, *ipso*-C.

4-(4-chlorophenyl)-6-(4-morpholinophenyl)py-rimidin-2-amine (12)

IR (KBr) (cm^{-1}): 3396, 3217, 3027, 2962, 2920, 1656, 1229, 930, 819, 684; ^{1}H NMR (δ ppm): 3.35-3.39 (t, 4H, N(CH$_2$)$_2$, J = 4.7 Hz), 3.89-3.91 (t, 4H, O(CH$_2$)$_2$, J = 4.7 Hz), 5.28 (s, 2H, NH$_2$), 7.26-7.85 (m, 9H, H$_{arom}$), the signal for H-5 proton may be merged with the aromatic protons; ^{13}C NMR (δ ppm): 47.6 N(CH$_2$), 66.5 O(CH$_2$), 104.5 C-5, 163.8 C-2, 164.1 C-6, 164.7 C-4, 127.0-139.1 −C$_{arom}$, 141.5,

152.5. *ipso*-C.

4-(4-methoxyphenyl)-6-(4-morpholinophenyl)-pyrimidin-2-amine (13)

IR (KBr) (cm^{-1}): 3447, 3200, 2972, 2922, 1659, 1600, 1243, 929, 821, 607; ^{1}H NMR (δ ppm): 3.32-3.35 (t, 4H, N(CH$_2$)$_2$, J = 4.6 Hz), 3.86 (s, 3H, OCH$_3$), 3.87-3.90 (t, 4H, O(CH$_2$)$_2$, J = 4.8 Hz), 5.26 (s, 2H, NH$_2$), 7.18-7.82 (m, 9H, H$_{arom}$), the signal for H-5 proton may be merged with the aromatic protons; ^{13}C NMR (δ ppm): 46.7 N(CH$_2$), 55.0 OCH$_3$, 67.3 O(CH$_2$), 103.9 C-5, 163.7 C-2, 164.7 C-6, 165.0 C-4, 127.5-142.1 −C$_{arom}$, 153.8, 154.2 *ipso*-C.

4-(4-fluorophenyl)-6-(4-morpholinophenyl)pyrimidin-2-amine (14)

IR (KBr) (cm^{-1}): 3434, 3200, 2967, 2923, 1624, 1599, 1226, 928, 815, 645; ^{1}H NMR (δ ppm): 3.34-3.36 (t, 4H, N(CH$_2$)$_2$, J = 4.7 Hz), 3.87-3.88 (t, 4H, O(CH$_2$)$_2$ J = 4.8 Hz), 5.29 (s, 2H, NH$_2$), 7.30-8.03 (m, 9H, H$_{arom}$), the signal for H-5 proton may be merged with the aromatic protons; ^{13}C NMR (δ ppm): 46.6 N(CH$_2$), 67.3 O(CH$_2$), 103.5 C-5, 163.8 C-2, 164.2 C-6, 164.4 C-4, 126.5-140.0 −C$_{arom}$, 140.6, 154.0 *ipso*-C.

4-(4-bromophenyl)-6-(4-morpholinophenyl)pyrimidin-2-amine (15)

IR (KBr) (cm^{-1}): 3398, 3219, 3029, 2965, 2922, 1659, 1231, 934, 821, 687; ^{1}H NMR (δ ppm): 3.36-3.39 (t, 4H, N(CH$_2$)$_2$, J = 4.8 Hz), 3.87-3.91 (t, 4H, O(CH$_2$)$_2$, J = 4.8 Hz), 5.29 (s, 2H, NH$_2$), 7.29-7.87 (m, 9H, H$_{arom}$), the signal for H-5 proton may be merged with the aromatic protons; ^{13}C NMR (δ ppm): 47.8 N(CH$_2$), 66.6 O(CH$_2$), 104.8 C-5, 163.9 C-2, 164.3 C-6, 164.8 C-4, 127.5-139.7 −C$_{arom}$, 152.6, 141.6 *ipso*-C.

4-(4-morpholinophenyl)-6-(3-nitrophenyl)pyrimidin-2-amine (16)

IR (KBr) (cm^{-1}): 3400, 3200, 3060, 2961, 2920, 1661, 1566, 1229, 928, 824, 776, 697; ^{1}H NMR (δ ppm): 3.36-3.39 (t, 4H, N(CH$_2$)$_2$, J = 4.6 Hz), 3.88-3.92 (t, 4H, O(CH$_2$)$_2$, J = 4.6 Hz), 5.28 (s, 2H, NH$_2$), 7.27-8.28 (m, 9H, H$_{arom}$), the signal for H-5 proton may be merged with the aromatic protons; ^{13}C NMR (δ ppm): 47.3 N(CH$_2$), 67.6 O(CH$_2$), 104.4 C-5, 163.8 C-2, 164.2 C-6, 164.4 C-4, 125.2-131.5 −C$_{arom}$, 146.7, 153.9 *ipso*-C.

4-(3-chlorophenyl)-6-(4-morpholinophenyl)pyrimidin-2-amine (17)

IR (KBr) (cm^{-1}): 3398, 3219, 3028, 2963, 2923, 1655, 1228, 929, 817, 686; ^{1}H NMR (δ ppm): 3.36-3.38 (t, 4H, N(CH$_2$)$_2$, J = 4.6 Hz), 3.88-3.90 (t, 4H, O(CH$_2$)$_2$, J = 4.7 Hz), 5.29 (s, 2H, NH$_2$), 7.16-7.74 (m, 9H, H$_{arom}$), the signal for H-5 proton may be merged with the aromatic protons; ^{13}C NMR (δ ppm): 47.7 N(CH$_2$), 66.6 O(CH$_2$), 104.4 C-5, 163.6 C-2, 164.3 C-6, 164.8 C-4, 126.2-138.8 −C$_{arom}$, 146.5, 152.3, *ipso*-C.

4-(3-fluorophenyl)-6-(4-morpholinophenyl)pyrimidin-2-amine (18)

IR (KBr) (cm^{-1}): 3437, 3204, 2965, 2928, 1623, 1597, 1225, 921, 811, 649; ^{1}H NMR (δ ppm): 3.33-3.35 (t, 4H, N(CH$_2$)$_2$, J = 4.7 Hz), 3.87-3.88 (t, 4H, O(CH$_2$)$_2$, J = 4.8 Hz), 5.27 (s, 2H, NH$_2$), 7.28-8.05 (m, 9H, H$_{arom}$), the signal for H-5 proton may be merged with the aromatic protons; ^{13}C NMR (δ ppm): 46.8 N(CH$_2$), 67.4 O(CH$_2$), 103.7 C-5, 163.9 C-2, 164.4 C-6, 164.5 C-4, 125.9-140.3 −C$_{arom}$, 146.6, 154.2 *ipso*-C.

Microbiology
Materials

All the clinically isolated bacterial strains namely *Bacillus subtilis, Bacillus cerues, Micrococcus luteus* and *Salmonella typhii* and fungal strains namely *Aspergillus niger, Candida 6* and *Candida 51* were obtained from Faculty of Medicine, Annamalai University, Annamalainagar-608 002, Tamil Nadu, India.

In vitro antibacterial and antifungal activity

MIC in µg/mL values was carried out by two-fold serial dilution method.[30] The respective test compounds 10-18 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 Sabourauds 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 1.1-1.5×10^2 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 $37 \pm 1\,^{\circ}C$ for bacteria and $28 \pm 1\,^{\circ}C$ for fungi. MICs were recorded by visual observations after 24 h (for bacteria) and 72 - 96 h (for fungi) of incubation. Penicillin was used as standard for bacteria studies and Amphotericin B was used as standards for fungal studies.

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