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Microwave Mediated Synthesis And Evaluation Of Some Novel Pyrimidines For Antimicrobial Activity

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Abstract: The synthesis and antimicrobial evaluation of some novel pyrimidine derivatives 3(a-j) are synthesized by Claisen-Schmidt condensation of arylketones and 4-hydroxy-3-methylbenzaldehyde to give respective chalcone and further cyclised with guanidine in ethanol using microwave irradiation in lesser time with higher yields. All the compounds were characterized by physical and spectral data. The compounds were screened for anti-microbial activities. Compounds 3j & 3i were found to possess significant anti-bacterial activity against both gram positive and gram negative bacteria at the tested concentrations when compared with that of standard drug ampicillin. In anti-fungal study, compounds 3h, 3j and 3b have exhibited significant anti fungal activity when compared with standard drug fluconazole. These compounds can be further exploited to get the potent lead compound.
Key word: Pyrimidines, Microwave, Antimicrobial activity.

Introduction

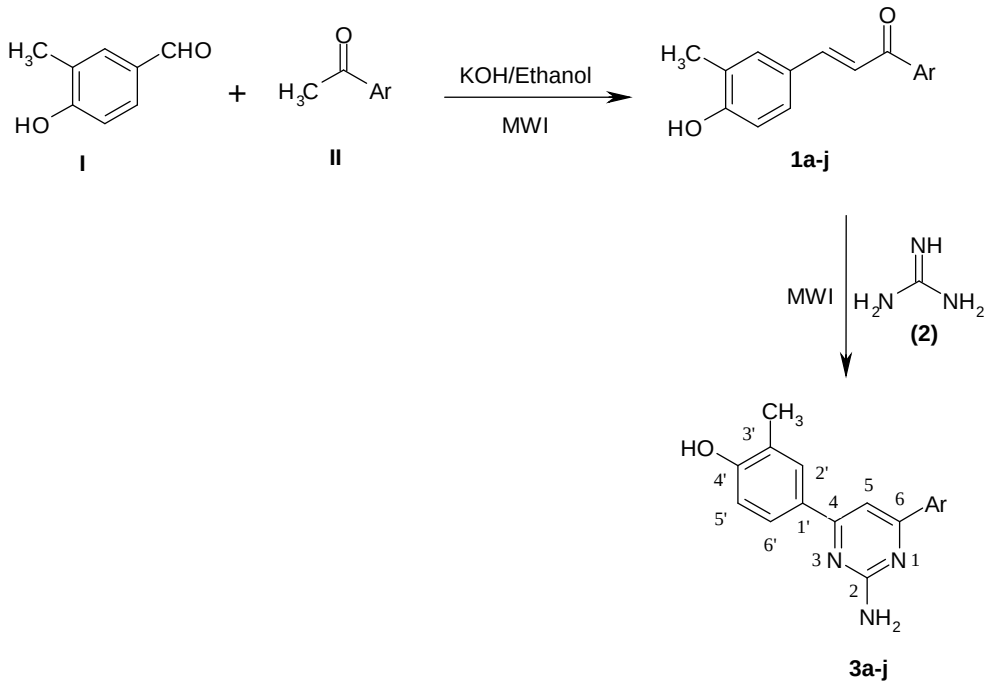
In the recent years, microwave assisted organic reactions have emerged as a new tool in organic synthesis. Important advantages of this technique include highly accelerated rate of the reaction, reduction in reaction time with an improvement in the yield and quality of product. Moreover, this technique is considered as an important approach towards green chemistry because of its eco-friendly nature. Large number of heterocyclic compounds being used as therapeutic agents and these compounds are also essential for the human life. Pyrimidines and its derivatives are also an important class of heterocyclic compounds because of their broad range of biological activities such as antibacterial [1-3], antimalarial [4], antiviral [5-6], anti-inflammatory [7-8] and anticancer [9-11]. As a part of our research interest in the development of multifunctional libraries of pyrimidines, therefore it was felt worthwhile to study these reactions under microwave irradiation with the aim of decreasing the reaction time and increasing the yield **(Scheme 1)** and also evaluate the antimicrobial activity of synthesized compounds.

Experimental

All the chemicals and solvents used were of synthetic grade obtained from Sd Fine chemicals and AVRA labs. Completion of the reactions was monitored time to time by analytical thin layer chromatography (TLC) using E-Merck 0.25mm silica gel plates. Visualization was accomplished with UV light (256nm) and iodine chamber. The purity of compounds was checked by a single spot in TLC and solvent system for TLC was determined on trial & error basis. All the melting points were determined in open capillaries, using Boitus melting point apparatus, expressed in ^{o}C and are uncorrected. All the IR spectra were recorded on SCHIMADZU FT-IR SPECTROPHOTOMETER by using 1 % potassium bromide discs. The Electronic Spray

Ionization mass spectra were recorded on Agilent 1100 series. The ^{1}H NMR spectra of the compounds were recorded on Bruker AMX 400 MHz NMR spectrophotometer using TMS as an internal standard and the values are expressed in δ ppm.

Scheme 1:



General procedure for the synthesis of chalcones by Claisen-Schmidt condensation method (1a-j):

Equimolar quantities (0.005 mol) of 4-hydroxy-3-methylbenzaldehyde and respective arylketones were mixed and dissolved in minimum amount of alcohol. To this, aqueous potassium hydroxide solution (50%, 7.5 ml) was added slowly and mixed well and irradiated in the domestic microwave for 3 min at 100% intensity. Completion of the reaction was identified by TLC using silica gel-G. After completion of the reaction, the mixture was poured onto crushed ice, acidified if necessary with dilute hydrochloric acid, and the solid that separated was isolated by filtration, dried and purified by column chromatography on silica gel (100- 200 mesh, Merck), with a mixture of ethyl acetate and hexane as the mobile phase.

General procedure for the synthesis of pyrimidines:

To a mixture of chalcone (1a-j) (0.01 mol), guanidine hydrochloride (2) (0.01 mol) and ethanol (30mL) were taken into a 100 mL conical flask. This reaction flask was irradiated in domestic microwave for 3-5min at 100% intensity. Completion of the reaction was established by TLC using silica gel-G. After completion of the reaction, the reaction mixture was poured onto crushed ice with constant stirring. The solid that separated was filtered, dried and purified by column chromatography on silica gel, using a mixture of ethyl acetate and hexane as the mobile phase. The purified pyrimidine derivatives were obtained as light to bright yellow fine powders (3a-j).

Antimicrobial activity:

All the synthesized compounds **3a-j** were screened for their antibacterial activity against *Staphylococcus aureus* (NCIM-2079), *Bacillus subtilis* (NCIM-2063), *Escherichia coli* (NCIM-2068) and *Proteus vulgaris* (NCIM-2027) by serial tube dilution technique [12-13] using ampicillin as reference standard, and antifungal activity against *Aspergillus* niger (ATCC-6275) and *Candida tropicalis* (ATCC-1369) by using fluconazole as reference standard. The observed minimum inhibitory concentrations (MIC) values for all the synthesized compounds are presented in Table 4.

Results And Discussion

Chemistry:

Synthesis of 3-(4-hydroxy-3-methylphenyl)-1-(pyridin-2-yl) prop-2-en-1-one (1a):

A mixture of 4-hydroxy-3-methylbenzaldehyde (0.005 mol) and 1-(pyridin-2-yl)ethanone (0.005 mol) was stirred in ethanol (7.5 ml) and then an aqueous solution of potassium hydroxide (50%, 7.5 ml) was added to it. The reaction mixture was irradiated in microwave oven for 3 min at 100% intensity. The progress of reaction was monitored by TLC using ethylacetate and hexane as mobile phase then the reaction mixture was acidified with 1:1 HCl and H2O. The solid separated out was filtered under vacuum and washed with water, purified by column chromatography and crystallized from a mixture of ethyl acetate and hexane. Homogeneity of the compound was checked by TLC.

Compound **1a** analyzed for $C_{15}H_{13}NO_2$, m.p 188-191^{0}C and well supported IR spectrum showed the characteristic intense absorption bands at 3350 (O-H), 1655(C=O), 1585 (C=N), 1580 (C=C quadrant of Ar) and 1504 (CH=CH).

The ^{1}H NMR spectrum (400MHz, $CDCl_3$) of compound **1a** showed the characteristic signals of CO-CH= and =CH-Ar at δ 7.83 and 7.96 as doublets. The spectrum also showed an aromatic methyl group as a singlet at δ 2.19 integrating for three protons. The peaks in between 6.93 to 8.63 integrating for seven protons were due to the aromatic hydrogens.

By adopting the above synthetic procedure, compounds **1b** to **1j** were also synthesized. All these compounds are new and the characteristic physical data were presented separately in table 1.

Synthesis of 4-(2-amino-6-(pyridin-4-yl)pyrimidin-4-yl)-2-methylphenol (3b):

3-(4-hydroxy-3-methylphenyl)-1-(pyridin-4-yl)prop-2-en-1-one **(1b)** (0.001 mol) was condensed with guanidine hydrochloride (0.001 mol) in the presence of potassium hydroxide (0.002 mol) in absolute ethanol (5 ml) under microwave irradiation for 4min at 100% intensity. The solvent was evaporated *in vacuo* and crushed ice was added to the residue while mixing thoroughly, whereupon a bright yellow solid separated out. This solid was filtered under vacuum, dried and purified by column chromatography to give pure pale yellow solid.

The compound **3b** was analyzed for molecular formula as $C_{16}H_{14}N_4O$, m.p. 168^{0}C, well supported by a $[M+H]^+$ ion at *m/z* 279.10 in its positive mode electrospray ionization mass spectrum. IR spectrum (cm^{-1}) showed the characteristic bands at 3360 (NH_2), 3352 (O-H), 1600 (C=N) and 1504 (C=C).

The ^{1}H NMR spectrum of compound **3b** showed the characteristic C-5-H of the pyrimidine around δ 7.8 as singlet and C-2-NH_2 at 5.4 as singlet. The spectrum also accounted for the other aromatic protons of the hetero aromatic and phenyl rings in between δ 6.91-8.61. By adopting the above synthetic procedure various 2-amino pyrimidines were synthesized. The physical and spectral characteristics of selected pyrimidines were presented separately in table 2 and 3.

Table 1: Physical data of chalcones (1a-j)

Compound	Ar	Formula	Melting Point (^{0}C)	Yield (%)
1a	2"-Pyridyl	$C_{15}H_{13}NO_2$	188	71
1b	4"-Pyridyl	$C_{15}H_{13}NO_2$	278	66
1c	4"-Chlorophenyl	$C_{16}H_{13}ClO_2$	260	67
1d	2"-Pyrrolyl	$C_{14}H_{13}NO_2$	117	66
1e	4"-Hydroxyphenyl	$C_{16}H_{14}O_2$	210	70
1f	2"-Furyl	$C_{14}H_{12}O_3$	225	70
1f	4"-Nitrophenyl	$C_{16}H_{13}NO_4$	120	64
1h	2"-Thienyl	$C_{14}H_{12}SO_2$	282	66
1i	3", 4", 5"-Trimethoxy phenyl	$C_{19}H_{20}O_5$	155	68
1j	2"-Indolyl	$C_{18}H_{15}NO_2$	173	71

Table 2: Physical data of 2-aminopyrimidines (3a-j)

Compound	Ar	Formula	Melting Point (^{0}C)	Yield (%)
3a	2"-Pyridyl	$C_{16}H_{14}N_4O$	166	70
3b	4"-Pyridyl	$C_{16}H_{14}N_4O$	168	69
3c	4"-Chlorophenyl	$C_{17}H_{14}N_3ClO$	192	72
3d	2"-Pyrrolyl	$C_{15}H_{14}N_4O$	210	69
3e	4"-Hydroxyphenyl	$C_{17}H_{15}N_3O_2$	196	72
3f	2"-Furyl	$C_{15}H_{13}N_3O_2$	232	68
3g	4"-Nitrophenyl	$C_{17}H_{14}N_4O_3$	117	72
3h	2"-Thienyl	$C_{15}H_{13}N_3SO$	265	74
3i	3", 4", 5"-Trimethoxy phenyl	$C_{20}H_{21}N_3O_4$	123	67
3j	2"-Indolyl	$C_{19}H_{16}N_4O$	178	72

Table 3. ^{1}H NMR data of compounds (3a-j)

Compound	Chemical shift (δ) in ppm
3a	8.28 (1H, s, C-5-H), 5.40 (2H, s, C-2-NH2), 7.87 (1H, s, C-2'-H), 2.32 (3H, s, C-3'-CH3), 6.89 (1H, d, C-5'-H),7.9 (1H, d, C-6'-H), 8.57 (1H, d,C-3"-H), 7.67 (1H, t, C-4"-H),7.47 (1H, m, C-5"-H), 8.68 (1H, d, C-6"-H)
3b	7.86 (1H, s, C-5-H), 5.40 (2H, s, C-2-NH2),7.83 (1H, s, C-2' -H), 2.33 (3H, s, C-3'-CH3), 6.91(1H, d, C-5'-H), 7.94 (1H, d, C-6'-H), 8.61 (1H, d, C-2"-H), 8.09 (1H, d, C-3"-H), 8.09 (1H, d, C-5"-H), 8.61(1H, d, C-6"-H)
3c	7.8 (1H, s, C-5-H), 5.44 (2H, s, C-2-NH2), 7.77 (1H, s, C-2'-H), 2.32 (3H, s, C-3'-CH3), 6.89 (1H, d, C-5'-H), 8 (1H, d, C-6'-H), 6.87 (1H, d, C-3"-H), 6.18 (1H, m, C-4"-H), 6.69 (1H, d, C-5"-H)
3d	7.81 (1H, s, C-5-H), 5.42 (2H, s, C-2-NH2),7.84 (1H, s, C-2'-H), 2.32 (3H, s, C-3'-CH3), 6.89 (1H, d, C-5'-H), 7.94 (1H, d, C-6'-H), 7.99 (1H, d, C-2"-H), 7.6 (1H, d, C-3"-H), 7.6 (1H, d, C-5"-H), 7.99 (1H, d, C-6"-H)
3i	7.57 (1H, s, C-5-H), 5.44 (2H, s, C-2-NH2),7.84 (1H, s, C-2'-H), 2.32 (3H, s, C-3'-CH3), 6.89 (1H, d, C-5'-H), 7.94 (1H, d, C-6'-H), 7.61(1H, s, C-2"-H), 3.93 (3H, s, C-3"-OCH3), 3.94 (3H, s, C-4"-OCH3), 3.93 (3H, s, C-5"-OCH3),7.61 (1H, s, C-6"-H)
3j	7.38 (1H, s, C-5-H), 5.44 (2H, s, C-2-NH2), 7.86 (1H, s, C-2'-H), 2.32 (3H, s, C-3'-CH3), 6.89 (1H, d, C-5'-H),7.91 (1H, d, C-6'-H), 8.21 (1H, m, C-2"-H), 8.6 (1H, d, C-4"-H), 7.05 (1H, t, C-5"-H), 7.22 (1H, t, C-6"-H), 7.44 (1H, d, C-7'-H)

Table 4. Antimicrobial activity of 2-aminopyrimidine derivatives (3a-j).
(Expressed as MIC in µg/mL)

Antimicrobial activity						
Compound	***B.subtilis***	***S.aureus***	***E.coli***	***P.vulgaris***	***A.* niger**	***C. tropicalis***
3a	256	256	256	256	256	256
3b	256	256	256	256	128	128
3c	128	256	256	256	256	256
3d	128	256	128	128	256	256
3e	256	-	-	-	-	256
3f	128	128	64	128	256	128
3g	256	256	-	-	256	128
3h	64	256	128	128	16	16
3i	32	64	128	64	256	128
3j	16	32	32	32	32	16
Ampicillin	<1	<1	<1	<1	-	-
Fluconazole	-	-	-	-	<2	<2

(-) indicates MIC > 256

All synthesized pyrazoles **(3a-j)** have been evaluated for their antibacterial activity against *E.coli*, *P.vulgaris* (Gram-negative) and *S.aureus* and *B.subtilis* (Gram-positive) using serial tube dilution method. The results of this evaluation compared with ampicillin as reference standard.

From the above results it is evident that all the pyrimidines showed antibacterial activity with different MIC values against the tested organisms, but not comparable with that of the standard. Among the tested compounds **3j** (2-indolyl) and **3i** (3,4,5-trimethoxyphenyl) was found to be potent against *B. subtilis* with a MIC value of 16µg/mL and 32 µg/mL respectively. The compound **3f** (2-furyl) and **3j** (2-indolyl) was found to be moderately potent against *B. subtilis* with a MIC value of 128-256 µg/mL. The compound **3j** was active against *E. coli* with a MIC value of 32 µg/mL. The compounds **3i**, **3f** and **3h** were active against *P. vulgaris* with a MIC value of 32-128 µg/mL. The other compounds also exhibited activity with a MIC values ranging from 128-256 µg/mL.

Among all the compounds tested, compounds **3j** and **3i** possessed maximum activity which may be due to of hetero aryl moieties at C-6 position of pyrimidine and thus reveals the importance of such groups for favorable antibacterial activity. This also suggested that pyrimidines having more number of heteroaromatic rings when synthesized may demonstrate promising antibacterial activity. Infact, it was observed in the present study that C-6 substituted pyrimidine ring contributed favorably to the antibacterial activity.

iii) *Antifungal activity*:

The antifungal activity of pyrimidines **(3a-j)** have been evaluated against *A.niger* and *C.tropicalis* and fluconazole employed as reference standard by using serial tube dilution method.

A close examination of the antifungal data of pyrimidines revealed that some of the compounds in this series have been found effective against both fungi at 16 µg/mL concentration level when compared with reference standard fluconazole. Among the compounds tested for antifungal activity, compounds **3h** (2-thienyl) and **3j** (2-indolyl) and **3b** (4-pyridyl) found to be potent against *A. niger* with a MIC value of 16-128 µg/mL. All the other compounds showed activity with a MIC values ranging from 128-256 µg/mL which was less when compared to the activity of other compound tested.

Compounds **3h, 3j, 3b** and **3i** possessed maximum activity which may be due to the presence of 2-thienyl, 2-indolyl, 4-pyridyl and 3,4,5-trimethoxyphenyl pharmacophore at C-6 position of pyrimidine structure. This reveals the importance of the heteroccyclic rings in enhancing the antifungal activity. Moreover it has been found that compounds **3a, 3c, 3d** and **3e** also exhibited weak activity against both the fungi.

Conclusion

In the present study we have demonstrated a simple, efficient and cleaner strategy for the synthesis of 2-aminopyrimidines by reacting of different chalcones with guanidine in alcoholic KOH under microwave irradiation conditions. With encouraging antimicrobial activity results, all the synthesized compounds need to be evaluated in terms of active concentration and also examine the mechanism of compounds responsible for antimicrobial activity. All the synthesized compounds can be further explored for structural modifications and studies concerning the structure-activity relationships are in progress in our laboratory.

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