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Elucidation and Evaluation of Substituted Pyrimidines

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The present work deals with the synthesis and characterization of seven substituted pyrimidines from chalcones. The chalcones that are prepared from substituted acetophenones and aromatic aldehydes with guanidine hydrochloride. The newly synthesized compounds were elucidated by using physical and spectral characteristics and the structures were established. The compounds were evaluated for antimicrobial and antimycobacterial potentials using Kirby bauer method and Alamar blue assay technique.

Key Words: Chalcones, Amino pyrimidines, Elucidation, Physical data, Spectral data, Alamar blue assay.

INTRODUCTION

Medicinal chemistry concerns with the discovery, development, identification and interpretation of the mode of action of biologically active compounds at molecular level. Synthetic drugs have resulted by simple changes in the structural alignment of a few novel heterocyclic compounds. Chalcone is an aromatic ketone that forms the central core for a variety of important biological compounds. Due to the interesting activity of chalcone derivatives as biological agents, considerable attention has been focused on synthesizing this class of intermediates. The most general and widely used route to pyrimidine synthesis involves the combination of a reagent containing N-C-N skeleton with C-C-C unit[1-3].

The initial step involves the condensation of substituted acetophenones with various aromatic aldehydes in the presence of a strong base to form chalcones. The basic mechanism behind is Claisen Schmidt condensation. The cyclocondensation of chalcone with guanidine hydrochloride resulted in the formation of newer amino pyrimidines.

EXPERIMENTAL

Melting points were recorded in open glass capillaries electronically and are uncorrected. Purity of the compounds were checked by TLC on silica gel plates and spots were visualized by exposure to iodine vapours. The solvent system used for TLC was benzene:chloroform:methanol in the ratio 50:30:20.

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The IR spectra were recorded on thermoniolet FT-IR 200 spectrometer in KBr pellets. The ^{1}H NMR spectra on AMX 400M Hz in $CDCl_3$ using tetramethyl silane as internal standard. Compounds were screened for antibacterial activity by Kirby bauer method at PSG College of Pharmacy, Coimbatore. They were also evaluated for their antitubercular potentials by Alamar blue assay technique at Rajiv Gandhi Institute for Science and Technology, Trivandrum, India.

Preparation of benzylidene acetophenone (chalcone): A solution of 22.5 g of sodium hydroxide was dissolved in 200 mL of water. 122.5 mL of rectified spirit was taken, immersed in an ice bath, poured 52 g of freshly distilled acetophenone and stirred with a mechanical stirrer. Then 46 g of pure benzaldehyde was added and stirred vigorously till the mixture was thick, kept in the fridge overnight. The crude chalcone was then filtered and recrystallized from alcohol[4-6]. The yield of pure benzylidene acetophenone was 77 g (85 %). This substance should be handled with care since it acts as a skin irritant.

Preparation of substituted amino pyrimidine: Equimolar quantities of chalcone and guanidine hydrochloride were dissolved in ethanol. Double the quantity of sodium hydroxide was dissolved in minimum amount of water and added to reaction mixture. After 6 h reflux, it was poured into 250 mL of water and recrystallized[7,8].

Biological evaluation: The newly synthesized amino pyrimidines were screened for antibacterial activity using Kirby bauer method against *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli*. Dimethyl sulphoxide was used as the solvent and gentamycin as the standard[9,10].

The novel compounds were evaluated for their antitubercular potentials using Alamar blue assay technique. *Mycobacterium tuberculosis* H37 RV strain maintained on Lowenstein Jensen medium was used as test organism. Stock solutions of the newer compounds were prepared in dimethyl formamide, filtered, sterilized and were added to 450 µL of middle brook TB broth to achieve concentrations of 1, 0.5, 0.25 and 0.1 µg/mL. 50 mL of the vortexed culture was inoculated, mixed and incubated at 37 ºC using isoniazid as standard. On the seventh day, 25 µL of Alamar blue solution was added and observed for 6 h. Blue colour in the tube indicates sensitivity of *Mycobacterium tuberculosis* to the newly synthesized compounds and pink indicates resistance of the organism.

RESULTS AND DISCUSSION

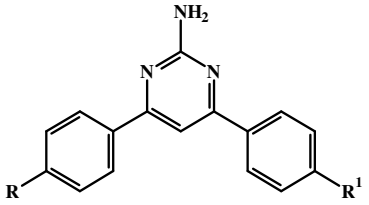
2-Amino-4,6 disubstituted pyrimidines were prepared from substituted acetophenones and aromatic aldehydes through an intermediate chalcone formation, in the presence of a strong base. Cyclocondensation of chalcones with guanidine hydrochloride resulted in the formation of substituted amino pyrimidines. The nomenclature of the synthesized compounds are given in Table-1.

The synthesized compounds were purified by recrystalization by using suitable solvents. The purity was checked with TLC. The physical characteristics are tabulated in Table-2. The Infrared spectral data[7,8] has been furnished in Table-3.

TABLE-1
NOMENCLATURE OF COMPOUNDS

Compound code	List of compounds synthesized
1	2-Amino-4,6-(diphenyl)pyrimidine
2	2-Amino-4-(phenyl)- 6 –(4-chlorophenyl)pyrimidine
3	2-Amino-4,6-(4,4' -di chloro phenyl)pyrimidine
4	2-Amino-4-(phenyl)-6-(4-methyl phenyl)pyrimidine
5	2-Amino-4,6-(4,4'-dimethyl phenyl)pyrimidine
6	2-Amino-4-phenyl-6-(4-methoxy phenyl)pyrimidine
7	2-Amino-4,6-(4,4'-methoxy phenyl)pyrimidine

TABLE-2
PHYSICAL DATA STUDIES OF NEWER PYRIMIDINES



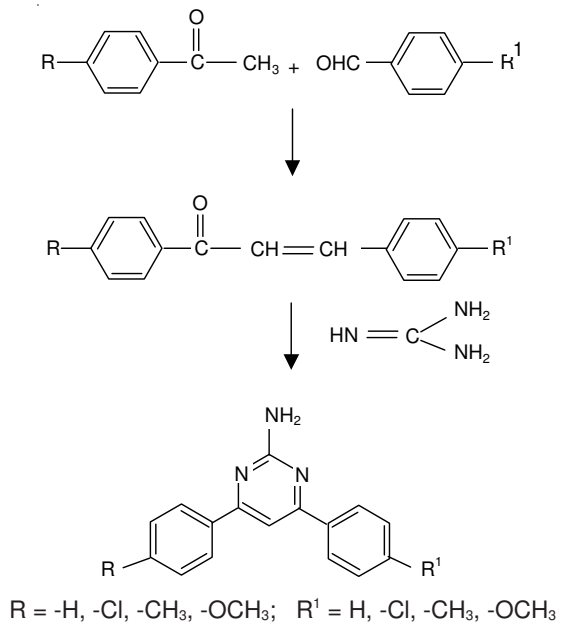
S. No.	R	R^1	m.f.	m.w.	m.p. (ºC)	Yield (%)	R_f	λ_{max}
1	-H	-H	$C_{16}H_{13}N_3$	247.0	143	76	0.66	266
2	-H	-Cl	$C_{16}H_{12}N_3Cl$	281.5	178	68	0.61	275
3	-Cl	-Cl	$C_{16}H_{11}N_3Cl_2$	316.0	174	62	0.68	270
4	-H	$-CH_3$	$C_{17}H_{15}N_3$	261.0	110	72	0.58	269
5	$-CH_3$	$-CH_3$	$C_{18}H_{17}N_3$	275.0	170	74	0.57	323
6	-H	$-OCH_3$	$C_{17}H_{15}N_3O$	277.0	117	65	0.63	269
7	$-OCH_3$	$-OCH_3$	$C_{16}H_{17}N_3O_2$	311.0	124	62	0.52	260

a. Recrystallization of above compounds: 1,2,3 = Ethanol, 4,5 = Benzene, 6,7 = Chloroform.
b. Solubility = Acetone, Dimethyl sulfoxide.
c. Solvent system for TLC = Benzene:Chloroform:Methanol (50:30:20)

TABLE-3
INFRARED SPECTRAL DATA (KBr)

S.N.	(Wave number/type of vibration cm^{-1})
1	3428($-NH_2$), 2923(Ar-CH), 1593(-CH=CH), 1448(C=N), 667(Sub. Ar)
2	3421($-NH_2$), 2851(Ar-CH), 1594(-CH=CH), 1489(C=N), 1091(-C-Cl)
3	1588(-CH=CH), 1489(C=N), 1091(-C-Cl)
4	3401($-NH_2$), 2920(Ar-CH), 1540(-CH=CH), 1446 (C=N), 1361(-C-CH_3)
5	3423($-NH_2$), 2917(Ar-CH), 1566(-CH=CH), 1511(C=N), 1329 (-C-CH_3)
6	3400($-NH_2$), 2921 (Ar-CH),1606(-CH=CH),1510(C=N),1248(-C-OCH_3)
7	3407($-NH_2$), 2923(Ar-CH), 1576(-CH=CH), 1509(C=N), 1237(-C-OCH_3), 1096(-C-Cl)

The PMR spectral data of compound **6** ($CDCl_3$) (δ ppm) 2.5 ($-CH_3$), 5.7 ($-NH_2$), 6-9 (-CH-Ar).



R = -H, -Cl, $-CH_3$, $-OCH_3$; R^1 = H, -Cl, $-CH_3$, $-OCH_3$

Scheme

TABLE-4
ELEMENTAL ANALYSIS OF COMPOUND-**6**

%	C	H	N
Calcd.	78.49	5.39	16.16
Found	78.40	5.36	16.11

TABLE-5
ANTITUBERCULAR ACTIVITY OF SUBSTITUTED 2-AMINO PYRIMIDINES

Comp. code	Concentration of compounds and colour developed				Analysis
1	P	P	P	P	Insensitive
2	B	B	P	P	Moderately sensitive
3	B	B	B	B	Sensitive
4	B	P	P	P	Insensitive
5	B	B	P	P	Moderately sensitive
6	B	B	B	B	Sensitive
7	B	B	B	B	Sensitive
Control	P	P	P	P	Insensitive
Blank	P	P	P	P	Insensitive
Std. (INH)	B	B	B	B	Sensitive

Antibacterial screening was carried out against *S. aureus, P. aeruginosa* and *E. coli* using gentamycin as the standard. But the synthesized compounds proved to be inactive even at a concentration of 500 µg/disc.

Evaluation to analyse antitubercular potentials of the synthesized compounds was carried out using Alamar blue assay method using isoniazid as the standard. Two compounds [(2-amino-4-(phenyl)-6-(4-chlorophenyl)pyrimidine, 2-amino-4,6-(4,4'-dimethylphenyl)pyrimidine)] possessed moderate sensitivity. But three compounds [(2-amino-4,6-(4,4'-dichloro phenyl)pyrimidine, 2-amino-4-phenyl-6-(4-methoxy phenyl)pyrimidine, 2-amino-4,6-(4,4'-methoxy phenyl)pyrimidine)] exhibited significant activity and proved to be equipotent with the standard drug isoniazid.

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