Research Article

SYNTHESIS, CHARACTERIZATION AND PHARMACOLOGICAL ACTIVITY OF NOVEL PYRIMIDINE ANALOGUES

Tribhuvan Singh*, Brijendra Kumar Soni, B.Vishnu Vardhan Reddy, Shalendra Bhandarkar, Vinod Kumar K.H.
Department of Medicinal Chemistry, M.E.S. College of pharmacy, Ardeshahalli Post, Bangalore, India.
*Corresponding author's E-mail: manjeet600@gmail.com

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ABSTRACT

A new series of pyrimidine derivatives have been synthesized by reacting corresponding chalcones with guanidine carbonate, followed by condensing the active hydrogen atom of 2-amino group of 4(4-substituted-phenyl)-6(furan-2-yl)-2-amino-pyrimidine with isonicotinic acid hydrazine/p-amino salicylic acid/5-amino tetrazole under microwave irradiation. The structures of three compounds were established by means of IR, ^{1}H-NMR. The title compounds were also screened for analgesic, anticonvulsant and antibacterial activities.

Keywords: Pyrimidine, Analgesic, Anticonvulsant, Antibacterial and Micro wave assisted organic synthesis.

INTRODUCTION

Appreciable number of six membered heterocyclic compound containing nitrogen atom, has been the subjected of study in the recent year. It plays a vital role owing to their wide range of biological and pharmacological activities. These were well known compounds, that were found to possess various biological activities like anti oxidant, anti allergic, hyperglycemic, vasodilation and β-adreno receptor, antagonistic[1-3], anti tubercular[4], anti helminthic, antimicrobial ant viral, anti malarial, anti cancer, anti platelet, anti thrombotic properties. It was also found that, they posses analgesic, anti inflammatory, anti convulsant, activity, antibacterial.

Epilepsy is the most frequent neurologic affection characterized by excessive temporary neuronal discharge. The overall prevalence's of the disease is 0.5-1.0% of the population and up to 50 million people worldwide. Many patients with epilepsy don't respond well to the currently available anti epileptic drugs (AED), such as phenytoin, carbamazepine, diazepam, phenobarbital, ethosuximide, valproate, valrocemide, vigabatrin, dalbapetin, zonisamide, lamotrigine, tiagabine, feibmate, retigabine, levertiracetom which are effective towards only 50-80% of the patients and present some undesirable side effects, such as vertigo ataxia, headache, hirsutism, hepato toxicity, gastro intestinal and cardiovascular side effects.

This study seeks to synthesize a series of novel pyrimidine derivatives in view of the continued interest synthetic routes for preparing heterocyclic systems. Various useful synthetic analogues with improved therapeutic properties can be obtained from single lead compound by structural modifications. We have attempted to synthesize some pyrimidine as derivatives and screened for their anti bacterial, analgesic and anti convulsant activities.

MATERIALS AND METHODS

The title compound melting points were taken by open capillary tubes and were uncorrected. The purity of compounds was checked by TLC on silica gel-G plates using methanol: chloroform (1:9) and acetone: chloroform (1:1) solvent system. IR spectra were recorded KBr plates on Shimadzu 8000 Series spectrophotometer, H^{1}-NMR spectra on a various EM/200, advance 200MHz spectrophotometer using DMSO as solvent TMS as internal standard (chemical shift values expressed in PPM).

Preparation of (1-(4-chloro-phenyl)-3-(furan-2-yl) propen-1-one chalcones (i)[5]

A mixture of 25g of NaoH, 200ml of water and 100g of (125.5ml) of rectified spirit was taken in a 500ml flask. The flask was immersed in an ice bath, provided with mechanical stirrer and to this 4 substituted (Cl, CH_3, Br & OCH_3) acetophenone was poured (0.43moles). The mixture was stirred and then 46g (44ml, 0.43moles) of pure 2-furaldehyde was added. The mixture temperature was kept at about 25°C (the limits as 15-30°C) and stirred vigorously with mechanical stirrer until the mixture is so thick that stirring is no longer effective (2-3hours). Then the mechanical stirrer was removed and left reaction mixture in an ice chest or refrigerator overnight. The product was filtered with suction on Buchner funnel and washed with cold water until the washings are neutral to litmus and then with 20ml of ice-cold rectified spirit. The crude chalcones was dried in the air and recrystallized from (5ml/g, warm 50°C) rectified spirit yield 82%, M.P 54-57°C)

Preparation of 4-(4-chloro-phenyl)-6-furan-2-yl-pyrimidine-2-yl amine (ii)

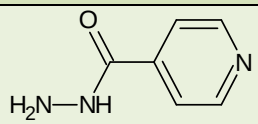
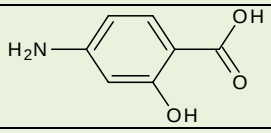
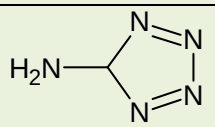
A mixture of 1-(4-chloro,bromo,methyl,methoxy-phenyl)-3-(furan-2-yl)propene-1-one chalcones-I (0.01mole) and guanidine carbonate (0.04moles) was heated under reflux

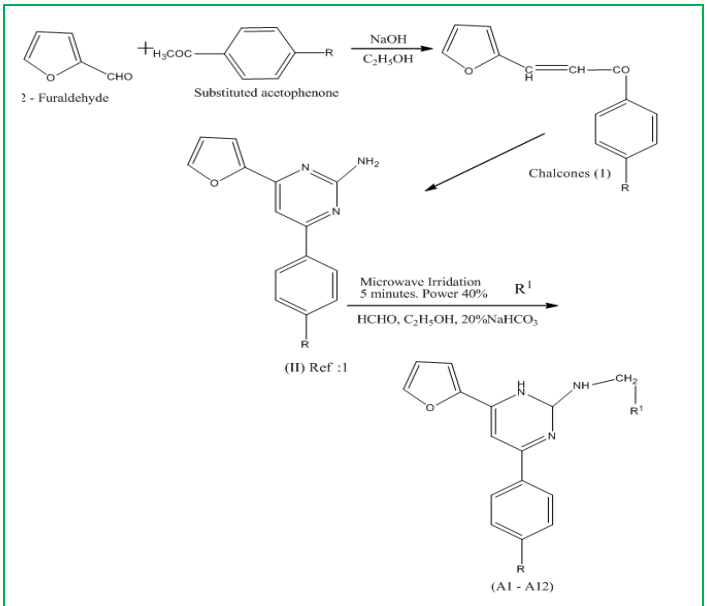
in (1ml) ethanol for 8hours. The contents were evaporated to dryness and the product so obtained was washed with water repeatedly and then recrystallized from ethanol. Yield 58%, M.P-192-194°C.

Synthesis of iso nicotinic acid N-{[4-(4-chloro-phenyl)-6-furan-2-yl-pyrimidine-2-ylamine]-methyl}-hydrazide(B1)[6]

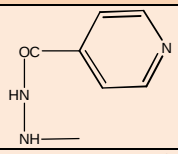
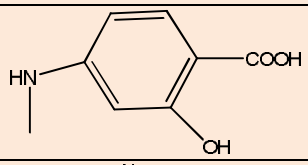
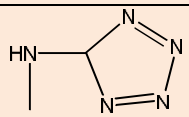
A mixture of 4-(4-chloro, bromo, methyl, methoxy-phenyl)-6-furan-2-yl pyrimidine-2-yl amine II (0.01mole), Iso nicotinic acid hydrazine (INH) (1.37g, 0.01moles), Formaldehyde (0.5ml), ethanol (1ml), $NaHCO_3$ (1ml of 20%) were taken in a mortar and made a homogenous paste using a pestle. The paste was transferred to a beaker and exposed to microwave irradiation for 5mins, at intervels of 30 secomds. After completion of the reaction (by running pre coated TLC), ice-cold water was added to the reaction mixture and precipitated solid was separated by filtration and recrystallized from ethanol to get Isonicotinic acid N-{[4-(4-chloro, bromo, methyl, methoxy-phenyl)-6-furan-2-yl pyrimidine-2-yl amino]-methyl}-hydrazide (A1), Yield 55% M.P-154-157°C. The compounds A2 to A12 were synthesized by following above similar procedure. The physical data of the synthesized compounds are shown in table No.1

Table 1: Physical data of synthesized compounds

S. No	Compound code	R	R^1	M.P (°C)	Mol.wt	Mol.Formula	% Yield
1	A_1	Cl		154-57	420	$C_{12}H_{17}ClN_6O_2$	55
2	A_2	Br		165-68	465	$C_{12}H_{17}BrN_6O_2$	62
3	A_3	Ome		164-86	416	$C_{22}H_{20}N_6O_3$	68
4	A_4	CH_3		148-50	400	$C_{22}H_{20}N_6O_2$	62
5	A5	Cl		162-65	436	$C_{22}H_{17}ClN_4O_4$	63
6	A6	Br		178-81	481	$C_{22}H_{17}BrN_4O_4$	60
7	A7	Ome		165-67	432	$C_{23}H_{20}N_4O_5$	58
8	A8	CH_3		155-57	416	$C_{23}H_{20}N_4O_4$	59
9	A9	Cl		161-63	370	$C_{16}H_{15}ClN_5O$	56
10	A10	Br		171-73	414	$C_{16}H_{15}BrN_8O$	63
11	A11	Ome		153-55	366	$C_{17}H_{18}N_8O_2$	60
12	A12	CH_3		173-75	350	$C_{17}H_{18}N_8O$	58



SCHEME

Compound	R	R^1
A1	Cl	
A2	Br	
A3	Ome	
A4	CH_3	
A5	Cl	
A6	Br	
A7	Ome	
A8	CH_3	
A9	Cl	
A10	Br	
A11	Ome	
A12	CH_3	

Acute toxicity studies[7]

The acute toxicity of synthesized compound was determined by using albino mice of either sex (20-30gm) maintained under standard husbandary conditions. The animals were fasted overnight prior to the experiment and fixed dose (OECD) guide line no.425 method of (CPCSEA) was adopted for toxicity studies. Effective dose (ED_{50} –therapeutic dose) is taken as $1/10^{th}$ of lethal dose.

Analgesic activity[8]

The analgesic activity of all the synthesized compound's were carried out by Eddy's hot plate method using albino mice's of either sex (20-30g), animals were deprived of food for 18hours. Prior to experiment [the first group was control group and received standard pentazocine drug (10mg/kg IP) and the remaining groups reward the compounds under investigation at 200mg/kg P.O].

All the synthesized compounds (200mg/kg P.O) concentration were subjected to the determination of analgesic activity and compared the basal reaction time in seconds at different time intervals against the drug pentazocine (10mg/kg) and gum acacia 2% was used as control. The results are shown in table no.2.

Anti-convulsant activity[9]

The anti-convulsant activity of all the synthesized compounds were carried out by MES induced convulsions method using albino mice's of either sex (20-30gm) animals were deprived of food for 18hours. Prior to experiment [the first group was control group and received standard phenytoin drug (10mg/kg IP) and the remaining groups reward the compounds under investigation at 200mg/kg P.O]

A 60 mA current was delivered transauricularly for 0.2seconds in mice via small alligator clips attached to cornea for recording various parameters. Mice were placed in a rectangular plastic cage with an open top, permitting full view of animal's motor response to seizure in the pilot study of various phases of convulsions. The following parameters were recorded during 30min test session. Tonic flexion, tonic extension, clonus convulsions, percent protection. The values were expressed as mean ± SEM from 6 animals. The results were subjected to statistical analysis by using ANOVA followed by Tukell-kramer test to calculate the significance difference if any among the groups $P<0.05$ was considered as significant. The results are shown in table No.4.

Anti-bacterial activity[10]

Anti-bacterial activities of all synthesized compounds were determined by the disc diffusion method against the gram +ve organism. Bacillus subtills and Bacillus pumilis and gram –ve organisms E-coli, pseudomonas aeruginosa at 100mg/ml concentration. The bacteria's were subcultured in nutrient agar medium. The petri dishes were incubated at 37°C for 24hours. Standard antibacterial drug ampicillin at 100mg/ml concentration was also increased under similar conditions. The results are shown in table No.5

RESULTS AND DISCUSSION

Analgesic activity

The synthesized pyrimidine derivatives A1, A2, A3and A4 were selected for the screening of analgesic activity at the dose of 2mg/kg using Eddy's hot plate method in albino mice. The results are shown in table no. 3. The basal reaction time of compounds A1, A2, A3 and A4 are 14.94, 14.71, 13.60 and 11.16 seconds respectively at 60 minutes, where the standard drug pentazocine (10mg/kg) used was 13.87seconds at 60mins. From the above data it is cleared that former 6 compounds were having equipotent analgesic activity and later two compounds are having moderate to less analgesic activity when compared to that of standard drug pentazocine. From the analgesic activity evaluation data it is cleared that compounds A1, A2 and A3 were shown very good analgesic activity where as the compound A4 was shown less analgesic activity when compared to that of standard as well as other pyrimidine derivatives.

Anti Convulsant activity

The pyrimidine derivatives were selected and screened for the anticonvulsant activity at the dose of 200 mg/kg using MES induced model in albino mice. The anticonvulsant activity data is given in the table No.4. The anticonvulsant activity result of the synthesized pyrimidine was studied by considering two main parameters:

1) Lesser duration of tonic extensor phase

2) Maximum protection of animals after 24 hrs.

The compounds A1, A2 and A3 were exhibited significant anticonvulsant activity against electrically induced convulsions. The protected animal's up to 60.66% and the tonic extensor phase of these agents are 5.93, 9.19 and 9.65 seconds respectively. Whereas rest of the compounds thought they showed good decrease in the tonic extensor phase phenyl to in exerts its anticonvulsant action against generalized tonic seizures by preventing seizure spread.

From anticonvulsant evaluation it was found that significant anticonvulsant activity of A1, A2 and A3 may be due to the presence of electron withdrawing groups at R position of the phenyl ring.

Antibacterial Activity

The synthesized 12 compounds were screened for the Antibacterial activity studies at 50µg/mL and 100µg/mL using DMSO as a control against staphylococcus aureus, Bacillus pumilis, Bacillus subtilis, Escherichisa coli and Pseudomonas aeruginosa by disk-diffusion method on nutrient agar media, Ampicillin was used as standard drug for the comparison at the concentration 50µg/mL and 100µg/mL against Gram-positive and Gram-negative bacteria used for the study.

Data in the Table.No-5 clearly indicates that none of the compound exhibits antibacterial activity. The zone of inhibition of all the synthesized compounds were between 7-10 mm at 50µg/mL concentration and 11-13 mm at 100µg/mL concentration. Whereas the zone of inhibition of standard drug Ampicillin was 21-24 mm at 50µg/mL concentration and 32-35 mm 100µg/mL concentration, many studies have revealed that pyrimidine's are having good antibacterial activity, but in the present study none of the synthesized compound exhibits such anti bacterial activity. This may be due to the bulky substitution at R^1 position of benzene pyrimidine, which may be leads to the rigidity of the compounds and this may hinders the cleavage of molecules in physiological pH, which is a basic requirement for the activity of pyrimidine derivatives.

Table 2: Analgesic activity data of pyrimidine derivatives

Treatment	No.of Animals	Avg.wt of animals (g)	Dose (mg/kg)	Basal reaction time(sec) after							
				0 sec	20sec	40sec	60sec	80sec	100sec	120sec	180sec
Control (gum acacia)	6	34	---	3.90±0.367	4.12±0.473	4.12±0.553	4.27±0.324	4.27±0.324	3.88±0.372	5.20±0,72	---
Standard (pentazocine)	6	28	0.28	6.53±0.889	12.87±1.332	12.91±1.320	13.87±0.851	13.65±0.851	11.83±1.042	12.33±0.918	13.14
A1	6	30.66	6.13	3.97±0.058	10.73±1.597	12.45±1.613	14.94±0.449	14.15±0.449	14.54±0.290	11.91±1.052	14.03
A2	6	29.33	5.86	5.49±o.416	11.66±1.889	14.66±0.210	14.71±0.605	13.39±0.605	14.09±0.573	11.79±1.028	10.49
A3	6	32	6.4	5.62±o.158	12.13±1.813	13.40±1.008	13.60±1.164	13.16±1.164	13.16±1.164	12.97±0.028	12.19
A4	6	28	5.6	5.46±0.717	9.92±1.346	12.51±1.576	11.16±1.084	11.72±1.084	11.72±1.084	13.36±0.519	---

Table 3: IR/^{1}H NMR spectral study of the synthesized compounds

S.no	IR(cm^{-1})	^{1}H-NMR(δ ppm)
A1	3383(N-H str),299(C-H Ar.str),2850(C-H str),1667(C=O Amide str.),1592(C=N str.),879(C=H Ar.def.)	----------
A2	3384(N-H str),1680(C=N str.),1580(C=N str),1432(O-H def.),1167(C-O str.),913(C-H Ar.def.)	----------
A3	3383(N-H str.),2990(C-H Ar.str.),2850(C-H str.),1667(C=O Amide str.),1592(C=N str.),1254 and 1170(C-O str.),879(C-H Ar.def)	7.44-7.80(m,12H, Ar-H),3.89 (S,3H,OCH_3),3.79(S,2H,CH_2)
A4	3437(N-H str.),2926(C-H Ar.str.),2858(C-H str.),1663(C=O Amide str.),1550(C=N str.),1165(C-O str.),845(C-H Ar.def.),3394(N-H str.),2840(C-H Alkane.str),1669(C=O Acid.str)	----------
A5	1578(C=N str.),1498(O-H def.),1174(C-O str.),870 and 831(C-H Ar.def.),	----------
A6	3387(N-H str.),2811(C-H Alkane.str.),1666(C=O Acid str.),1557(C=N str.),1405(O-H def.),1173(C-O str.),885(C-H Ar.def)	7.23-7.66(m,11H,Ar-H),6.12 (S,H,NH),4.94(S,2H,CH_2)
A7	3383(N-H str.),2990(C-H Ar.str.),2850(C-H str.),1667(C=O Amides str.),1592(C=N str.),879(C-H Ar.def.)	----------
A8	3384(N-H str.),2800(C-H Alkane str.),1680(C=O Acid str.),1580(C=N str.),1432(O-H def.),1167(C-O str.),913 and 880(C-H Ar. Def.)	----------
A9	3380(C-H Ar.str.),3409(N-H str.),1676(C=C Ar.str.),1581(C=N str.),1490(C-H Alkane str.),1148(C-O str.),819(C-H Ar.def.)	7.12-7.64(M,8H,Ar-H),4.98(S,2H,CH_2), 4.50(S,2H,NH)
A10	3392(N-H str.),2804(C-H Ar.str.),1674(C=C Ar.str.),1579(C=H str.),1486(C-H Alkane str.),1169(C-O str.),885(C-H Ar.def.)	----------
A11	3980(C-H Ar.str.),3409(N-H str.),1676(C=C Ar.str.),1581(C=N str.),1490(C-H Alkane str.),1148(C-O str.),819(C-H Ar.def.)	----------
A12	3392(N-H str.),2804(C-H Ar.str.),1674(C=C Ar.str.),1579(C=H str.),1486(C-H Alkane str.),1169(C-O str.),885(C-H Ar.def.)	----------

Table 4: Anticonvulsant activity data of pyrimidine derivatives

Group No	Treatment	Avg.wt	Avg.Dose (mL)	Latency (onset of clonus) (sec/30 min) mean±SEM	Duration of tonic extensor (sec/30 min) mean±SEM	% Production (24 hrs.)
I	Control (2% gum acacia)	26.75	0.26	3.62±0.036	13.35±0.018	12.5
II	Standard (phenytoin 25 mg/kg)	22.25	0.22	10.25±0.036	No	100
III	Compound A1 (200mg/kg P.O)	28.00	0.33	11.83±0.450	9.19±0.26**	66.66
IV	Compound A2 (200mg/kg P.O)	27.33	0.32	12.78±1.061	9.65±0.191**	66.66
V	Compound (200mg/kg P.O)	28.00	0.33	7.65±0.413	5.93±0.810**	66.66
VI	Compound One way ANOVA	26.00	0.30 F Df	7.67±0.602 12.912 59	5.02±2.058** 31.428 59	

n=6; Significance at P<0.05*,<0.05*,<0.001*** and ns-not significant.

Table 5: Antibacterial activity of newly synthesized pyrimidine derivatives

Sample code	Inhibition zone diameter in nm							
	B.subtills		B.pumills		E.coli		P.aureaginosa	
	50µg	100 µg	50µg	100 µg	50µg	100 µg	50µg	100 µg
A1	8	13	7	7	7	10	8	9
A2	7	10	8	7	8	11	8	10
A3	9	12	7	7	9	10	7	9
A4	8	9	8	8	8	13	7	11
A5	8	11	7	8	9	10	8	11
A6	9	10	7	10	9	9	9	12
A7	8	12	7	9	8	10	9	10
A8	8	13	7	9	9	11	9	11
A9	9	12	8	11	9	11	8	11
A10	10	12	8	8	9	12	7	11
A11	11	9	7	8	8	10	7	13
A12	11	9	8	8	8	12	8	11
Ampicillin	22	34	21	32	22	35	24	34
DMSO	-	-	-	-	-	-	-	-

*Average of triplicate ± standard deviation; Note: " _" denotes no activity, 7-9 mm better activity,10-13 mm moderate activity.

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REFERENCES

1. Ozair A, Mohd I, Khan SA, Synthesis and biological activity of some pyrimidine derivatives. Indian J Heterocyclic chem. 14:2005; 293-6
2. Ramesh D, Chandrashekar, Vaidya VP. Synthesis, analgesic, anti inflammatory and anti microbial activities of naptho [2,1-b] furan incorporated substituted pyrimidine's. Indian J Heterocyclic chem. 15:2006; 319-22
3. Fathalla OA, Radwan HH, Awad SM, Mohnaed MS, Synthesis and biological activity of new pyrimidine derivatives. Indian J of chem. 45B:2006; 980-5.
4. AL Barry. The antimicrobial susceptibility test: Principle and practices, edited by IIlus leu and Febiger (Philadelphia, Pa, USA), Biol. Abstr. 64:1976, 25783.
5. Fumiss BS, Hannaford AJ, Smith PWG, Patchel AR, Vogels Text book of practical organic chemistry. 5th ed.singapore: Pearson education (Singapore) pvt Ltd; 1996.
6. Shashikanth R.pattan, Khade AB, Khade MA, synthesis and antimicrobial evaluation of some new pyrimidine derivatives. Indian J.pharma.Res 43(3):2009; 308-12.
7. OECD 2001-guidelines on acute oral toxicity (AOT) Environmental health and safety monograph series on testing adjustment no.425.
8. Woolf G, Mac Donald AD.J. pharmacol EXP Ther,80:1944,300-3071944.
9. SW inyard EA Brown WC, Goodman L.SJ. pharmacol EXP Ther.,106:1952,319-330,1952.
10. Cruckshank, Duguid JP, Marmoin BP and swam HA. The practice of medical microbiology 12th edition, Chrechill livingstone, London,2:1957,190.

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