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Review Article

NEWER APPLICATIONS OF 2,4,6-TRISUBSTITUTED PYRIMIDINES AS POTENTIAL CYTOTOXIC AGENTS

Gaddam Vijaya Bhaskar Rao[1*], Zaheda Banu[1] and Hari Jagannadha Rao G[2]

[1]*Guntur Medical College, Guntur, Andhra Pradesh, India.
[2]Ashran Medical College, Eluru, Andhra Pradesh, India.

ABSTRACT

A number of 2,4,6-trisubstituted pyrimidines were reported to possess diverse biological activities like antimicrobial, antidepressant, analgesic, anti-inflammatory, anticancer, antiviral, antileishmanial, antituberculosis, anti-HIV and antimalarial activities. In view of the varied biological and pharmacological importance of pyrimidine derivatives, it is felt worthwhile to evaluate them for possible activities. These compounds therefore were screened for anticancer activity theIC-50 values of synthesized compounds revealed that the compounds having dihalogen substitution (B_6P_6 and B_5P_5) were found to be more potent than the compounds having electron releasing substituents. Pyrimidines with monohalogen substitution also enhanced the anticancer activity. Among all the compounds, compounds with fluorine substitution significantly enhanced the activity followed by compounds with chloro substitution.

Key Words: Pyrimidines, Cytotoxic etc.

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Corresponding Author

Gaddam Vijaya Bhaskar rao
Guntur Medical College, Guntur, Andhra Pradesh, India.

Email:- vijay057pharma@yahoo.co.in

INTRODUCTION

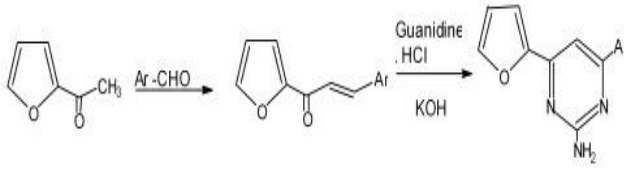
Pyrimidine is a six-membered heterocycle with two nitrogen atoms situated in a 1,3-arrangement. It is also known as *m*-diazine or 1,3-diazine. Both nitrogen atoms are like the pyridine nitrogen (Maayan S *et al.*, 2005; Nowakowska N, 2007; Go ML *et al.*, 2005). A number of 2,4,6-trisubstituted pyrimidines were reported to possess diverse biological activities like antimicrobial, antidepressant, analgesic, anti-inflammatory, anticancer, antiviral, antileishmanial, antituberculosis, anti-HIV and antimalarial activities (Elizabeth P *et al.*, 2006). In view of the varied biological and pharmacological importance of pyrimidine derivatives, it is felt worthwhile to evaluate them for possible activities. These compounds therefore were screened for anticancer activity (Tsukiyama RI *et al.*, 2002; Machodo TB *et al.*, 2005; Rao NR *et al.*, 2004).

MATERIALS AND METHODS

General procedure for the synthesis of 2,4,6-trisubstituted pyrimidines Synthesis of 2-amino-4-(2'-furyl)-6-(3",4",5"-trimethoxyphenyl) pyrimidine (b_1p_1)

1-(2 -furyl)-3-(3",4",5"-trimethoxyphenyl)-2-pro pen-1-one (B_1) (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) at reflux temperature on a water bath for 3 hrs. 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. Physical characterization data of 2,4,6-trisubstituted pyrimidines were given in table-1,spectral data were given in table-2 and 3



BIOLOGICAL EVALUATION

Anticancer activity

Methodology

The synthesized pyrimidines have been screened for anticancer activity on prostate cancer cell lines (DU-145) using MTT based cytotoxicity assay.

Chemicals/Biochemicals used in the present study

(a) Media: DMEM (Dulbecco's Modified Eagles Medium)
(b) 10 % Fetal bovine serum (FBS)
(c) MTT reagent : [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]
(d) Cell lines: DU-145 cell lines, were obtained from the National Centre for Cell Science (NCCS), pune (India)

Procedure

This method is based on a colorimetric assay which takes into account the ability of a mitochondrial dehydrogenase enzyme from viable cells to cleave the tetrazolium rings of the pale yellow MTT and form dark blue formazan crystals which is largely impermeable to cell membranes, thus resulting in its accumulation within healthy cells. The level of formazan created is a reflection of the number of surviving cells and shows a proportionality relationship between them.

The required cell proliferation assay kit was obtained from Roche Applied Sciences, Germany The procedure consists of seeding an equal number of DU-145 cells in each well of a 96- well microplate and incubating at 37°C in the presence of 5 % CO_2. Various concentrations of the test substances were added to the cells. For every 24 hrs the culture medium was renewed with the test substances. 0.5 % DMSO was added into the vehicle control culture wells. After 72 hrs treatment, 5 µl of MTT reagent (R&D systems USA) along with 45 µl of phenol red and FBS free DMEM (Sigma Life Science, USA) was added to each well and incubated for 4 hrs at 37°C in presence of 5 % CO_2. Then 50 µl of solublization buffer (R&D systems, USA) was added to each well to solubilize the coloured formazan crystals produced by the reduction of MTT. After 24 hrs the optical density was measured at 550 nm using a microplate reader (BioRad, USA). The results (mean O.D.± SD) obtained from quadruplicate wells were used in calculation to determine the IC_{50} of the test compounds.

The percent inhibition is then calculated from the formula:

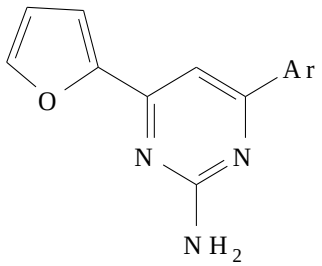
$$\% \text{ inhibition} = \frac{\text{Control O.D.} - \text{Sample O.D.}}{\text{Control O.D.}} \times 100$$

The dose levels of those pyrimidines which showed a minimum of 50 % inhibition (IC_{50}) were shown in table-4

RESULTS AND DISCUSSION

The IC-50 values for pyrimidines revealed that the compounds having dihalogen substitution (B_6P_6 and B_5P_5) were found to be more potent than the compounds having electron releasing substituents. Pyrimidines with monohalogen substitution also enhanced the anticancer activity. Among all the compounds, compounds with fluorine substitution significantly enhanced the activity followed by compounds with chloro substitution.

Table 1. Physical characterization data of 2,4,6-trisubstituted pyrimidines(B_1P_1-$B_{10}P_{10}$)



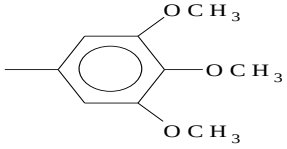
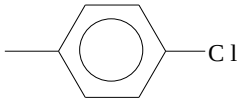
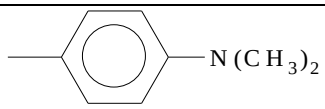
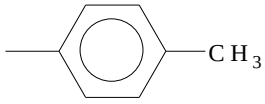
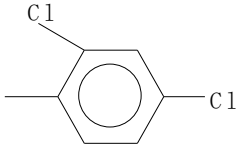
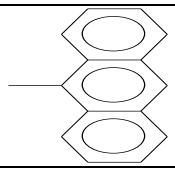
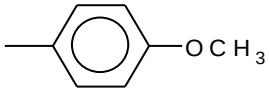
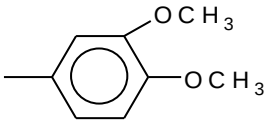
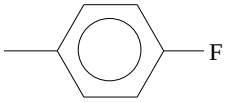
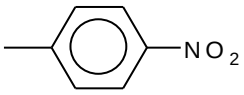
Compound	Structure	Molecular Formula	Relative Molecular Mass(RMM)	Melting Point (^{o}C)	Yield %
B_1P_1		$C_{17}H_{17}N_3O_4$	327	315-319	52
B_2P_2		$C_{14}H_{10}ClN_3O_2$	271	250-254	64
B_3P_3		$C_{16}H_{16}N_4O$	280	178-182	59
B_4P_4		$C_{15}H_{13}N_3O$	251	148-150	45
B_5P_5		$C_{14}H_9Cl_2N_3O$	325	143-147	57
B_6P_6		$C_{22}H_{15}N_3O$	337	238-240	71
B_7P_7		$C_{15}H_{13}N_3O_2$	267	313-317	62
B_8P_8		$C_{16}H_{15}N_3O_3$	297	223-225	67
B_9P_9		$C_{14}H_{10}FN_3O$	255	241-243	43
$B_{10}P_{10}$		$C_{14}H_{10}N_4O_3$	282	264-268	53

Table 2. IR spectral data (KBr disc) of 2,4,6-trisubstituted pyrimidines (B_1P_1-B_{10}P10)

Compound	Position of absorption band (cm^{-1})
B_1P_1	3414, 3380 (NH_2), 1591 (C=N), 1503 (C=C), 1387(C-N),1228 (C-O-C), 1178 (O-CH_3)
B_2P_2	3405, 3346 (NH_2), 1636 (C=N), 1578 (C=C),1383 (C-N), 858 (C-Cl)
B_3P_3	3410, 3332 (NH_2), 1610 (C=N), 1570 (C=C), 1391 (C-N),1178 (-N (CH_3)$_2$)
B_4P_4	3412, 3335 (NH_2), 1597 (C=N), 1520 (C=C), 1365 (C-N)
B_5P_5	,3410, 3326 (NH_2), 1605 (C=N), 1525 (C=C),1372 (C-N),892 (C-Cl)
B_6P_6	3413, 3328 (NH_2), 1632 (C=N), 1515 (C=C), 1375 (C-N)
B_7P_7	3414 (NH_2), 1598 (C=N), 1503 (C=C), 1366 (C-N),1225 (C-O-C)
B_8P_8	3320, 3187 (NH_2), 1597 (C=N), 1556 (C=C), 1354 (C-N), 1261(C-O-C)

B_9P_9	3468, 3318(NH_2), 1599 (C=N), 1510 (C=C), 1350(C-N), 1219 (C-F)
$B_{10}P_{10}$	3370 (NH_2), 1645 (C=N), 1557 (N=O, asymmetric), 1406 (C-N),1350 (N=O, symmetric)

Table 3. ^{1} H NMR spectral data (400MHz) of 2,4,6-trisubstituted pyrimidines ($B_1P_1 – B_{10}P_{10}$)

Compound	Chemical shift (δ) in ppm
B_1P_1	3.75-4.0 (9H, s, 3xOCH_3), 5.15 (2H, s, -NH_2), 6.45-6.60 (1H, m, C-4'-H) 7.38 (1H, d, J=6.0Hz, C-5'-H), 7.0 (1H, S ,C-5-H) 6.40 (2H, S , C-2"-H and C-6"-H), 7.28 (1H, d, J=6.0Hz C-3'-H
B_2P_2	5.45 (2H, s, -NH_2), 6.60 (1H, m , C-4'-H),8.03 (2H, d , J=8.0Hz, C-3"-H and C-5"-H),7.48 (2H, d, J=8.0Hz, C-2"-H and C-6"-H),7.62 (1H, d, J=6.5Hz and C-5'-H) 7.30 (1H, d, J=6.5Hz and C-3'-H), 7.40 (1H, S, C-5-H)
B_3P_3	3.10 (6H, s, -$N(CH_3)_2$), 5.20 (1H, s , -NH_2), 6.61 (1H, m , C-4'-H), 7.36 (1H, s , C-5-H),8.12 (2H, d , J=8.5Hz, C-3"-H and C-5"-H),6.78 (2H, d , J=8.5Hz, C-2"-H and C-6"-H),7.67 (2H, d, J=6.0Hz, C-3'-H and C-5'-H)
B_4P_4	2.46 (3H, s, Ar-CH_3), 5.25 (2H, s, -NH_2), 6.67 (1H, m, C-4'-H), 7.45 (1H, s, C-5-H), 8.06 (2H, d, J=8.0Hz, C-3"-H and C-5"-H), 7.36 (2H, d, J=8.0Hz, C-2"-H and C-6"-H), 7.71(1H, d, J=6.0Hz, C-5'),7.60 (1H, d, J=6.0Hz, C-3'H)
B_5P_5	5.78 (2H, s, -NH_2), 6.62 (1H, m, C-4'-H),7.62 (1H, s , -C-3"-H),7.64 (1H, d, J=6.5Hz, C-5'-H), 7.54 (1H, d, J=8.5Hz, C-5"-H),7.41 (1H, d, J=8.5Hz, C-6"-H), 7.39 (1H, d, J=6.5Hz, C-3'-H),7.35 (1H, s, C-5-H)
B_6P_6	5.85 (2H,s, -NH_2), 6.61 (1H,m, C-4'-H), 7.60 (1H,s, C-5-H) 8.06 (1H, d, J=6.0Hz, C-5'-H), 7.78 (1H, d, J=6.0Hz, C-3'-H) 7.22-7.55(9H, m, Ar-H)
B_7P_7	3.87 (3H, s, C-4"-OCH_3), 5.11 (2H, s, C-2-NH_2), 6.56 (2H, d, J=6.0 Hz, C-3' and 5'-H), 7.07 (2H, d, J=8.5 Hz, C-3"and 5"-H), 7.37 (1H, s, C-5-H), 7.58 (1H, s, C-2'-H), 8.05 (2H, d, J=8.5 Hz, C-2" and 6"-H)
B_8P_8	5.63 (2H, s, C-4'-NH_2), 5.21 (2H, s, C-2-NH_2), 6.64 (2H, d, J=6.5 Hz, C-3'), 7.19 (2H, dd, J=8.5 Hz, C-2" and 6"-H), 7.37 (1H, s, C-5-H),8.084 (2H, dd, J=8.5 Hz, J=8.5 Hz, C-3" and 5"-H)
B_9P_9	5.63 (2H, s, C-4'-NH_2), 5.21 (2H, s, C-2-NH_2), 6.64 (2H, d, J=6.5 Hz, C-3'), 7.19 (2H, dd, J=8.5 Hz, C-2" and 6"-H), 7.37 (1H, s, C-5-H), 8.084 (2H, dd, J=8.5 Hz, J=8.5 Hz, C-3" and 5"-H)
$B_{10}P_{10}$	5.22 (1H, s, C-2-NH_2), 6.64-6.65 (1H, t, C-4'-H),7.62-7.58 (2H, m, C-3'), 7.79 (2H, d, J=8.0Hz, C-2″ and 6″-H),7.88 (1H, d ,J=16Hz, -CO-CH=),8.24 (1H, d, J=15.6Hz, Ar-CH=),8.34 (2H, d, J=8.0Hz, C-3″and 5″-H)

Table 4. Anticancer Activity of trisubstituted pyrimidines (B_1P_1-$B_{10}P_{10}$), by MTT Assay on Human Prostate Cancer Cell Lines (DU-145)

Compounds	Ar	IC_{50}(µg/ml)
B_1P_1	4-methylphenyl	115
B_2P_1	4-fluorophenyl	24
B_3P_3	4-chlorophenyl	36
B_4P_4	2-chlorophenyl	59
B_5P_5	2,4-difluorophenyl	22
B_6P_6	2,4-dichlorophenyl	27
B_7P_7	3-nitrophenyl	86
B_8P_8	3,4,5-trimethoxyphenyl	120
B_9P_9	3-nitro-4-methylphenyl	92
$B_{10}P_{10}$	5-bromofuryl	39

CONCLUSION

The above IC-50 values for pyrimidines revealed that the compounds having dihalogen substitution (B_6P_6 and B_5P_5) were found to be more potent than the compounds having electron releasing substituents. Pyrimidines with monohalogen substitution also enhanced the anticancer activity. Among all the compounds, compounds with fluorine substitution significantly enhanced the activity followed by compounds with chloro substitution.

Pyrimidines having number of either of these substituents or both these substituents at different positions of the phenyl ring can be synthesized as the resulting compounds are likely to possess significant activity.

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CONFLICT OF INTEREST:
The authors declare that they have no conflict of interest.

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