RESEARCH PAPER

Received 28 February 1994
Indian J. Pharm. Sci., 1995, 57(6),pp.229-232

Synthesis and Antimicrobial Evaluation of Some Substituted Pyrido [2,3,- d]pyrimidines

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Synthesis of di-and trisubsituted pyrido[2,3-d]pyrimidines has been performed by the chemical transformation of 2,3,4,6,- tetrasubstituted pyridines with formamide, urea and different arlisocyanates. The authenticity of the products was established with the help of elemental analyses, IR and NMR spectral data. All of the synthesized compounds have antimicrobial activity.

A thorough surve of the literature reveals the vast expanse of the properties possessed by pyrido[2,3,-d]pyrimidines. They are being synthesized through many, but entirely different strategies[1-3] not only because of their immense biological and therapeutic implications[4-8] but also due to their utility as dye intermediates[9], fungicides[10] and other indispensable synthetic utilities[11,12]. Keeping the above conjucture in mind and in continuation of our earlier work[13], we wish to report, herewith, some newer pyrido[2,3- d]pyrimidines and their activity against microorganisms.

EXPERIMENTAL

Melting points were determined in open capillary tubes and are uncorrected. The IR spectra were recored by means of pressed KBr disks on a Perkin-Elmer 577 grating IR spectrophotometer. The ^{1}H NMR spectra were recorded in $CDCl_3$ & DMSO-d_6 on FX90Q Jeol type spectrometer at 90 MHz using TMS as the internal standard.

PREPARATION OF 2-AMINO-3-CYANO-4,6-DI-SUBSTITUTED PYRIDINES(II)

A mixture of substituted chalcone (0.01 mole), dicyanomethane (0.1 mole) and ammonium acetate (0.8 mole) in 100 ml ethanol was heated under reflux for 20-22 hours the contents were then cooled and poured into crushed ice, with constant stirring. The yellow suspension thus obtained was collected by filtration, washed with water, dried and crystallized from ethanol. In certain instances the yellow suspension turned into a sticky mass. This was triturated with petroleum ether 960-80°C) to obtain the compound (II) directly.

PREPARATION OF 4-AMINO-5,7-DISUBSTITUTED PYRIDO[2,3- D]PYRIMIDINES (III)

A mixture of II (0.01 mole) and formamide (0.04 mole) was refluxed on an oil bath for 15 hours. The reaction contents were cooled and poured into crushed ice. The compound (III) thus obtained was washed with water and crystallized from DMF-EtOH (1:2).

PREPARATION OF 4-AMINO-5,7-DISUBSTITUTED PYRIDO[2,3- D]PYRIMIDIN-2 (1H) ONES (V)

A mixture of II (0.01 mole) and urea (0.02 mole) was heated on an oil bath at 120-130°C for 2 hours. The temperature was gradually raised to 180°C (2 hrs) and finally the mixture was heated at 230°C for 2 hours. The compound V thus obtained was washed with water, saturated $NaHCO_3$ solution, finally with cold ethanol, dried and crystallized from DMF-EtOH (1:2).

*For Correspondence

PREPARATION OF 2-AMINO-3-CARBOXAMIDO-4,6 DISUBSTITUTED PYRIDINES (IV)

A mixture of II (2g) and 20% alcoholic potassium hydroxide (6g KOH in 30 ml of EtOH) was refluxed for about 8-9 hours. The contents were diluted with constant stirring. The resulting yellow coloured solid was collected by filtration, washed with water, dried and crystallized from ethanol.

PREPARATION OF 3,5,7-TRISUBSTITUTED PYRIDO[2,3-D]PYRIMIDIN-2,4(1h,3H)DIONES (VI)

A mixture of IV (0.01 mole), appropriate arylisocyanate (0.01 mole) and diphenyl ether (30 ml) was heated under reflux for 7-8 hours and then allowed to stand overnight at room temperature. The separated compound VI as solid was filtered out, washed with cold ethanol, dried and crystallized from DMF-EtOH(1:2).

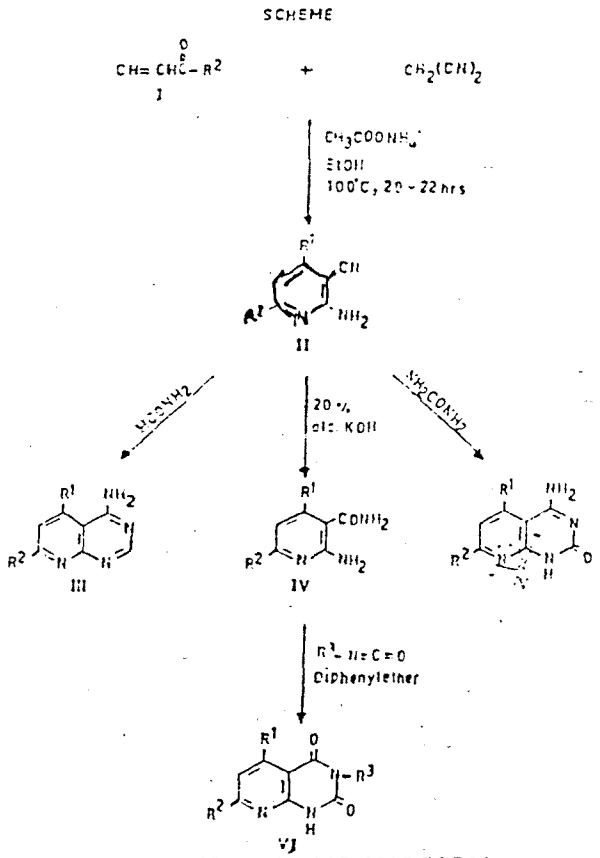
ANTIMICROBIAL EVALUATION

All of the synthesized compounds were put to two types of biological evaluations:

(1) Antibacterial and (2) Antifungal

Screening for both the types of activities was done at the concentration of 100 μg per disk using the paper disk method of Varma & Nobles[14]. Streptomycin and Mycostatin were used as the reference compounds for antibacterial and antifungal activities respectively. The test organisms are Staphylococcus aureus (gram positive bacterium), Escherichia coli (gram negative bacterium), Aspergillus niger, Aspergillus flavus, Alternaria tenuis, Curvularia lunata and Fusarium moniliformae (fungil). The results have been recorded in the form of activity indices i.e. the ratio of inhibition zone of the compounds to that of the standard compounds.

SCHEME



RESULTS AND DISCUSSION

α, β-unsaturated ketones, (1) were cyclized with dicyanomethane in 1:1 molar ratio in the presence of ammonium acetate to obtain 2-amino-3cyano-4,6-disubstituted pyridines (II). The compounds (II) were refluxed separately with formamide, 20% KOH and urea to obtain 4-amino- 5,7-disubstituted pyrido[2,3-d] pyrimidines (III), 2-amino-3- carboxamido-4, 6-disubstituted pyridines (IV) and 4-amino-5,7- disubstituted pyrido[2,3-d]pyrimidin-2(1H)-ones (V) respectively. The 2-amino-3-carboxamido-4,6-disubstituted pyridines (IV) were further condensed with different arylisocyanates into 3,5,7- trisubstituted pyrido-[2,3-d]pyrimidin-2,4(1H,3H)diones (VI) **(Scheme).** The structural assignments were made on the basis of element analysis, IR and ^{1}H NMR spectral studies. All of the synthesized compounds were screened against the bacteria E.coli & S. aureus and the fungi A. Niger, A. flavus, C. lunata, F. moniliformae & A.tenuis **(Table).** The method followed was that of Varma & Nobles[14].

The reported compounds were obtained in the form of coloured solids with high melting points. The

Table: Characterization data and activity indices of the title compounds

Compound No.	R_1	R_2	R_3	MP (°C)	Yield	Activity index*						
				%		Bacteria		Fungi				
						Ec	Sa	An	Af	At	Fm	Cl
IIIa	C_6H_5	3,4-$(OCH_3)_2C_6H_3$	—	>300	65	0.82	1.05	1.02	0.88	0.79	0.86	0.85
IIIb	4-ClC_6H_4	3,4-$(OCH_3)_2C_6H_3$	—	226-28	69	0.95	1.02	1.00	0.99	0.89	1.11	1.01
IIIc	3,4-$(OCH_3)_2C_6H_3$	4-FC_6H_4	—	134-36	66	1.14	1.13	1.08	1.11	1.06	1.13	1.16
Va	C_6H_5	3,4-$(OCH_3)_2C_6H_3$	—	212-14	72	0.9	1.14	1.05	0.90	0.84	0.84	0.94
Vb	4-ClC_6H_4	3,4-$(OCH_3)_2C_6H_3$	—	216-18	70	0.89	0.99	0.91	0.99	1.09	1.13	0.91
Vc	3,4-$(OCH_3)_2C_6H_3$	4-FC_6H_4	—	219-21	69	1.08	1.16	1.00	1.19	1.15	1.00	1.20
VIa	C_6H_5	3,4-$(OCH_3)_2C_6H_3$	C_6H_5	239-41	71	0.81	1.08	1.00	0.99	0.95	0.84	1.06
VIb	C_6H_5	3,4-$(OCH_3)_2C_6H_3$	3-ClC_6H_4	>300	63	0.86	1.15	1.04	1.11	1.09	10.88	0.91
VIc	4-ClC_6H_4	4-FC_6H_4	C_6H_5	242-44	66	1.26	1.19	1.15	1.30	1.15	1.10	0.94
VId	4-ClC_6H_4	4-FC_6H_4	3-ClC_6H_4	251-54	69	1.12	1.14	1.16	1.16	1.03	1.11	0.99
VIe	4-ClC_6H_4	4-OCH_3H_4	C_6H_5	215-17	70	1.00	1.08	1.06	1.10	0.86	0.96	0.94
VIf	4-ClC_6H_4	4-$OCH_3C_6H_4$	3-ClC_6H_4	198-200	70	1.03	0.96	1.04	0.99	0.89	0.93	0.96
VIg	3,4-$(OCH_3)_2C_6H_3$	C_6H_5	C_6H_5	>300	65	0.94	1.19	0.81	1.00	0.99	0.96	1.05
VIh	3,4-$(OCH_3)_2C_6H_3$	C_6H_5	3-ClC_6H_4	>300	67	0.88	1.06	0.96	0.87	0.89	0.86	0.95
VIi	3,4-$(OCH_3)_2C_6H_3$	4-ClC_6H_4	C_6H_5	>300	64	0.90	1.05	1.20	1.21	1.03	1.00	0.66
VIj	3,4-$(OCH_3)_2C_6H_3$	4-ClC_6H_4	3-ClC_6H_4	237-39	66	1.00	1.11	0.93	0.84	0.86	1.10	0.91

The compounds gave satisfactory elemental analysis.

*Activity index = inhibition area of the sample/inhibition area of the standard

**EC = Escherichia coli, Sa = Staphylococcus aureus,

An = Aspergillus niger, Af = Aspergillus flavus, At = Alternaria tenuis, Fm = Fusarium moniliformae, Cl = Curvularia lunata

proposed structures are well supported by the IR, ^1H NMR and ^{19}F NMR spectra.

In the IR spectra of 2-amino-3cyano-4,6-disubstituted pyridines (II), a sharp band was obtained at 2200 cm^{-1} which was completely absent in the spectra of the title compounds. The presence of the three characteristic bands of-NH_2 group in the spectra of the compounds (II), (III), (IV) & (V) in the region of 3445-3320 cm^{-1} and their disappearance from the spectra of (VI) further confirmed the completion of the reaction. NH group caused an absorption band in the region of 3140-3090 cm^{-1} in the spectra of the compounds (V) & (VI). The spectra of the compounds (IV), (V) and (VI) also exhibited a sharp band in the region of 1700-1675 cm^{-1} due to the presence of C = O group.

The ^1H NMR spectra of the compounds (III) & (V) showed a complex multiplet of aromatic and NH_2 protons in the region of δ 6.75-8.90 ppm. In the spectra of compounds (VI) aromatic protons caused a multiplet in the region of δ 6.58-8.92 ppm. NH proton of the compound (V) & (VI) appeared as a singlet in the range of δ 8.5-8.82 ppm. -OCH_3 protons caused a singlet in the region δ 3.45-4.15 ppm.

^{19}F NMR spectra of the F-containing compounds exhibited a sharp band in the region of δ -108.200 to - 116.100 ppm.

All the synthesized compounds when screened against the test microorganisms formed considerable zones of inhibition which were comparable to those of the reference compounds. A glance on the activity indices shows that the compounds (III_c), (V_c) & ($VI_{e,f,i,j}$), which possess electronegative substitutents such as fluoro or chloro, show activities better than those containing methoxy groups. The compounds (VI_c) & (VI_d) which had both the fluoro & chloro substitutents showed still better activities than others.

In conclusion it may be stated that the presence of electronegative groups enhances considerably the antimicrobial activities of the substituted Pyrido[2,3-d] pyrimidines.

ACKNOWLEDGEMENT

Authors are grateful of the Head, Chemistry department, University of Rajasthan, Jaipur for providing the necessary facilities. One of the authors (S.S) is also grateful to UGC for providing financial assistance.

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