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Preliminary communication

Synthesis of new 2-substituted pyrido[2,3-*d*]pyrimidin-4(1*H*)-ones and their antibacterial activity☆

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Abstract

2-Substituted-5,7-dimethyl pyrido[2,3-*d*]pyrimidin-4(1*H*)-ones (**8**) were synthesized by oxidation of 2-substituted-5,7-dimethyl dihydropyrido[2,3-*d*]pyrimidin-4(1*H*)-ones (**7**) which were in turn prepared from 2-amino-4,6-dimethyl nicotinamide (**5**) and substituted aryl aldehydes (**6**). 2-Amino-4,6-dimethyl nicotinamide (**5**) was prepared from ethyl cyanoacetate (**1**) via malonamamidine hydrochloride (**3**). The compounds were characterized by IR, NMR, MS and elemental analyses. Compounds **7** and **8** were screened for antibacterial activity against Gram positive and Gram negative bacteria. Dehydrogenated compounds (**8**) showed less antibacterial activity than the compounds **7**. Among all the test compounds screened for antibacterial activity **7c** (1.25 μg/ml) showed greater activity. All the synthesized compounds were found inactive when screened for antifungal activity at the concentration of 200 μg/ml.

Keywords: Pyrimidines; Pyrido[2,3-*d*]pyrimidines; Antibacterial activity; Nitrobenzene; Oxidation

1. Introduction

Pyrimidine analogues are used as chemotherapeutic agents for the treatment of diseases resulting from different microorganisms and now there has been a major expansion in the number of pyrimidine derivatives as drugs. The design of pyrimidine and purine analogues as antimetabolites involves changes in the substituents on ring, isosteric replacement of rings, changes in ring size, attached sugars and phosphate residues, etc. Many useful drugs containing these skeletal structures with diverse biological activities have now emerged and are widely used against tumors, viral diseases and found to possess antifungal, antibacterial and antiinflammatory activities.

Pyrimidine derivatives like pyrimethamine and trimethoprim are well known to inhibit the enzyme, dihydrofolate reductase (DHFR), thereby blocking the reduction to tetrahydrofolic acid from its dihydro precursor, an essential coenzyme in nucleic acid synthesis. Both of them exhibit a far higher affinity for protozoal than for mammalian DHFR, so that they are used for malarial prophylaxis without adversely affecting the human (host) enzyme which otherwise results in toxic effects. They are active against both *Plasmodium falciparum* and *Plasmodium vivax*, in combination with an appropriate sulfonamide. They are effective because the sulfonamide acts synergistically by arresting the production of dihydrofolic acid from *p*-aminobenzoic acid, thus achieving a double (sequential) blockade of the folate pathway in the microorganism, which (unlike man) cannot use preformed folic acid. Diaminopyrimidine derivatives trimetrexate, piritrexim and several fused pyrimidines and pyrido[2,3-*d*]pyrimidines

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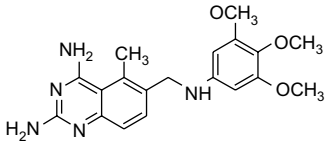
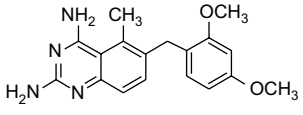
were synthesized and evaluated for antibacterial [1–3] and DHFR inhibition [4–8].



Trimetrexate



Piritrexim

Pyrido[2,3-*d*]pyrimidine ring system is present in a number of biologically active compounds which includes antitumor [9], antipyretic [10], analgesic [11], antihistaminic [12], PDE4 inhibitor [13], adenosine kinase inhibitor [14], tyrosine kinase inhibitor [15] and diuretic [16,17] activities. More specifically pyrido[2,3-*d*]pyrimidines were considered as inhibitors of *Pneumocystis carinii*, *Toxoplasma gondii* of tumor cells in culture. The activity is mainly due to inhibition of DHFR enzyme.

The synthesis of pyrido[2,3-*d*]pyrimidines is mainly by two ways, i.e. annulation of pyrimidine ring over pyridine or vice versa. The wide range of activity profile of pyrido[2,3-*d*] pyrimidines prompted us to probe the synthesis of novel analogues and to study their antibacterial, antifungal and cytotoxic activities.

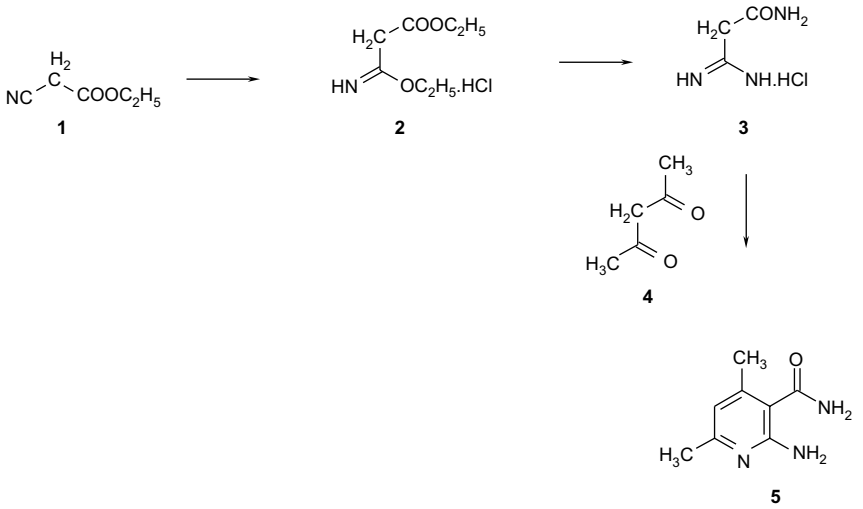
2. Chemistry

2-Amino-4,6-dimethyl nicotinamide (**5**) was prepared from ethyl cyanoacetate (**1**) via malonamamidine hydrochloride (**3**) (Scheme 1) [18,19]. On a reaction of **5** with aryl aldehydes (**6a–k**) in the presence of catalytic amounts of glacial acetic acid gave 2-substituted-5,7-dimethyl dihydropyrido[2,3-*d*]pyrimidin-4(1*H*)-ones (**7a–k**) in quantitative yields. Substituted aryl aldehydes (**6a–k**) were used as intermediates for the nucleophilic addition reaction with primary amines resulted in Schiff base formation, which spontaneously cyclised in acidic conditions to give various 2-substituted-5,7-dimethyl dihydropyrido[2,3-*d*]pyrimidin-4(1*H*)-ones (**7a–k**) (Scheme 2) [20,21]. Compound **5** in analogous reaction with heterocyclic aldehydes (**9a** and **9b**) gave respective dihydropyrido[2,3-*d*]pyrimidin-4(1*H*)-ones (**10a** and **10b**) (Scheme 3). The products were purified by passing through silica gel column using *n*-hexane, ethyl acetate (95:5) as mobile phase. They are characterized using physical, spectral (IR, ^{1}H NMR and MS) and elemental analyses. The IR spectra of compounds (**7** and **10**) showed signals around 3200 cm^{-1} (–NH) and 1620 cm^{-1} (C=O) and ^{1}H NMR spectra of compounds gave broad signal at δ 8.2–8.8 ppm indicating the presence of two –NH-protons. The proton of 2-position gave a prominent singlet at δ 5.8 ppm. The physical, spectral and analytical data of compounds **7** and **10** were presented in Table 1.

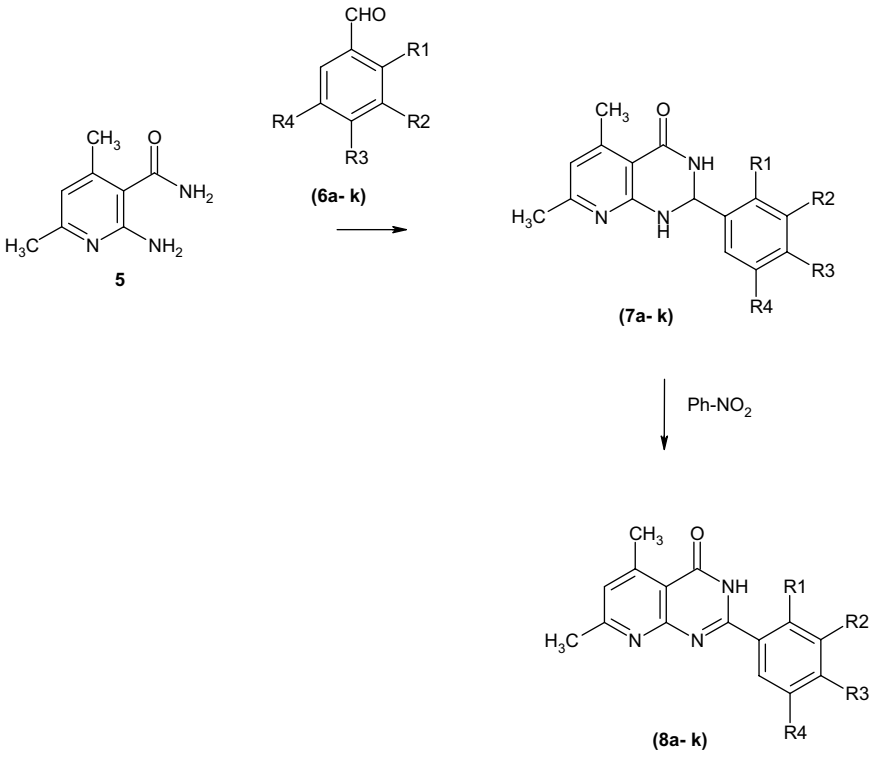
In the subsequent step different oxidation methods were attempted using potassium permanganate [22] and manganese dioxide [23,24], but the yields were poor. Taking the clue from the facile nature of oxidation process using nitrobenzene from literature [25], an attempt to oxidize **7** and **10** to 2-substituted-5,7-dimethyl pyrido[2,3-*d*]pyrimidin-4(1*H*)-ones (**8** and **11**) resulted in good yields (65–92%). The method was found to be superior (Scheme 4). A comprehensive account of the percentage yields of three different methods tried have been tabulated (Table 3). A mixture of 2-substituted-5,7-dimethyl dihydropyrido[2,3-*d*]pyrimidin-4(1*H*)-ones (**7** and **10**) and nitrobenzene was treated under reflux for 5 h. The ease of removal of nitrobenzene by steam distillation made it very facile. The structures of compounds **8** and **11** were deduced through physical, spectral (IR, ^{1}H NMR and MS) and elemental analyses. The formation of **8** was confirmed by the absence C-2 proton (at δ 5.8 ppm) was conspicuous in the ^{1}H NMR spectra of **8**. Further confirmation of the structure was through ^{13}C NMR of **8a** (2-C signal at δ 69.1 ppm disappeared). The physical, spectral and analytical data of compounds **8** and **11** were tabulated (Table 2).

3. Antibacterial assays (*in vitro*)

Four bacterial test organisms such as *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 96), *Escherichia coli* (MTCC 722) and *Klebsiella pneumoniae* (MTCC 109)

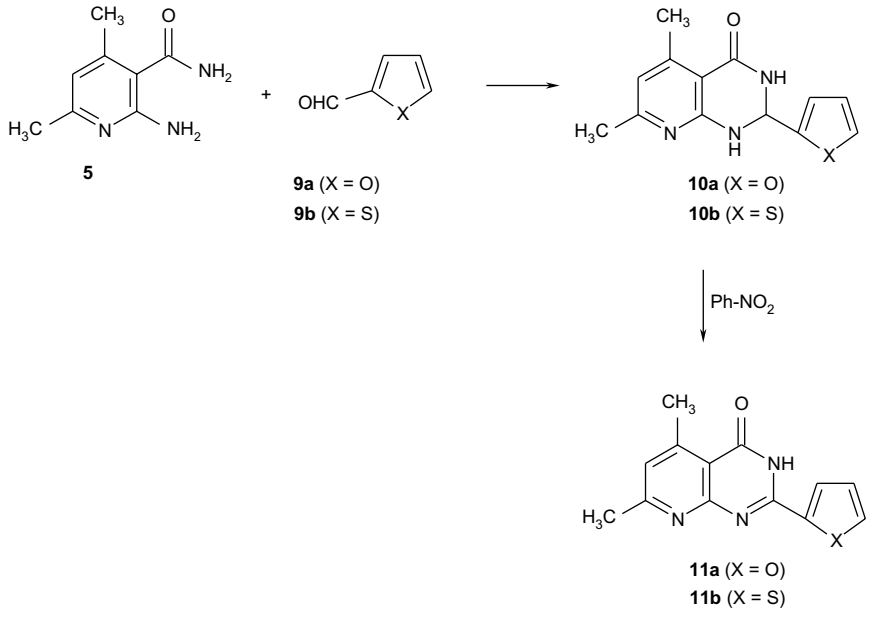


Scheme 1.



	R_1	R_2	R_3	R_4
a	H	OCH_3	OCH_3	H
b	H	H	OCH_3	H
c	H	OCH_3	OCH_3	OCH_3
d	OCH_3	H	OCH_3	H
e	Cl	Cl	H	H
f	CH_3	CH_3	H	H
g	H	H	OAc	H
h	H	OCH_3	OH	H
i	H	CH_3	OH	CH_3
j	OH	H	OCH_3	H
k	OH	H	H	H

Scheme 2.



Scheme 3.

Table 1
Physical, spectral and analytical data of 2-substituted-5,7-dimethyl dihydropyrido[2,3-*d*]pyrimidin-4(1*H*)-ones (**7** and **10**)

S. No.	Compound code	Mol. for. (Mol. wt.)	Melting point (°C) (yield %)	IR (cm^{-1}) KBr discs	^{1}H NMR ($CDCl_3$/DMSO-d_6) δ	Mass *m/z* (M^+)	CHN analyses found (calcd)
1	**7a**[a]	$C_{17}H_{19}N_3O_3$ (313)	190 (77.8)	3202 (NH) 1650 (C=O)	2.28 (s, 3H, CH_3), 2.6 (s, 3H, CH_3), 3.9 (s, 6H, OCH_3), 5.8 (s, 1H, H-2), 6.5 (s, 1H, H-6), 6.8–7.2 (m, 3H, Ar–H), 8.4 (s, br, 1H, NH, D_2O exchangeable), 8.7 (s, br, 1H, NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6, 400 MHz): δ (ppm) = 21.4 (CH_3); 24.5 (CH_3); 56.5 (2C), 69.1, 112.4, 112.9, 113.1, 115.4, 123.5, 140.1, 148.2, 150.2, 152.2, 153.4, 161.2, 168.3	314	C, 65.49, H, 6.43, N, 13.12 (C, 65.15, H, 6.12, N, 13.42)
2	**7b**	$C_{16}H_{17}N_3O_2$ (283)	209 (79)	3202 (NH) 1640 (C=O)	2.28 (s, 3H, CH_3), 2.6 (s, 3H, CH_3), 3.9 (s, 3H, OCH_3), 5.8 (s, 1H, H-2), 6.5 (s, 1H, H-6), 6.9–7.5 (d, 4H, Ar–H), 8.3 (s, br, 1H, NH, D_2O exchangeable), 8.52 (s, br, 1H, NH, D_2O exchangeable)	284	C, 67.49, H, 6.43, N, 14.82 (C, 67.81, H, 6.05, N, 14.84)
3	**7c**	$C_{18}H_{21}N_3O_4$ (343)	212 (86)	3219 (NH) 1662 (C=O)	2.2 (s, 3H, CH_3), 2.6 (s, 3H, CH_3), 3.8 (s, 9H, OCH_3), 5.8 (s, 1H, H-2), 6.5 (s, 1H, H-6), 6.8 (s, 2H, Ar–H), 8.3 (s, br, 1H, NH, D_2O exchangeable), 8.6 (s, br, 1H, NH, D_2O exchangeable)	344	C, 62.69, H, 6.51, N, 12.53 (C, 62.95, H, 6.17, N, 12.24)
4	**7d**	$C_{17}H_{19}N_3O_3$ (313)	189 (77.8)	3202 (NH) 1640 (C=O)	2.28 (s, 3H, CH_3), 2.6 (s, 3H, CH_3), 3.9 (s, 6H, OCH_3), 5.9 (s, 1H, H-2), 6.5 (s, 1H, H-6), 6.8–7.2 (m, 3H, Ar–H), 8.5 (s, br, 1H, NH, D_2O exchangeable), 8.7 (s, br, 1H, NH, D_2O exchangeable)	314	C, 65.29, H, 6.25, N, 13.22 (C, 65.15, H, 6.12, N, 13.42)
5	**7e**	$C_{15}H_{11}N_3OCl_2$ (320)	218 (77.1)	3200 (NH) 1620 (C=O)	2.28 (s, 3H, CH_3), 2.6 (s, 3H, CH_3), 5.8 (s, 1H, H-2), 6.6 (s, 1H, H-6), 6.9–7.5 (d, 3H, Ar–H), 8.2 (s, br, 1H, NH, D_2O exchangeable), 8.5 (s, br, 1H, NH, D_2O exchangeable)	322	C, 55.98, H, 3.25, N, 13.32 (C, 56.25, H, 3.46, N, 13.14)
6	**7f**	$C_{17}H_{19}N_3O$ (281)	192 (75)	3208 (NH) 1620 (C=O)	2.2 (s, 6H, CH_3), 2.5 (s, 3H, CH_3), 2.8 (s, 3H, CH_3), 5.8 (s, 1H, H-2), 6.4 (s, 1H, H-6), 7.4 (s, 3H, Ar–H), 8.3 (s, br, 1H, NH, D_2O exchangeable), 8.6 (s, br, 1H, NH, D_2O exchangeable)	282	C, 72.89, H, 6.45, N, 14.82 (C, 72.57, H, 6.80, N, 14.94)
7	**7g**	$C_{17}H_{17}N_3O_3$ (311)	192 (75)	3202 (NH) 1608 (C=O)	2.2 (s, 3H, CH_3), 2.8 (s, 3H, CH_3), 4.2 (s, 3H, OCH_3), 5.7 (s, 1H, H-2), 6.6 (s, 1H, H-6), 6.9–7.5 (d, 4H, Ar–H), 8.4 (s, br, 1H, NH, D_2O exchangeable), 8.6 (s, br, 1H, NH, D_2O exchangeable)	312	C, 65.29, H, 5.25, N, 13.82 (C, 65.57, H, 5.51, N, 13.50)
8	**7h**	$C_{16}H_{17}N_3O_3$ (299)	204 (72)	3200 (NH) 1600 (C=O)	2.28 (s, 3H, CH_3), 2.6 (s, 3H, CH_3), 4.0 (s, 3H, OCH_3), 5.8 (s, 1H, H-2), 6.5 (s, 1H, H-6), 6.9–7.4 (d, 3H, Ar–H), 8.5 (s, br, 1H, NH, D_2O exchangeable), 8.7 (s, br, 1H, NH, D_2O exchangeable)	300	C, 64.26, H, 5.56, N, 14.32 (C, 64.19, H, 5.73, N, 14.04)
9	**7i**	$C_{17}H_{19}N_3O_2$ (287)	198 (85)	3208 (NH) 1602 (C=O)	2.2 (s, 6H, CH_3), 2.5 (s, 3H, CH_3), 2.8 (s, 3H, CH_3), 5.8 (s, 1H, H-2), 6.5 (s, 1H, H-6), 6.9 (s, 2H, Ar–H), 8.3 (s, br, 1H, NH, D_2O exchangeable), 8.6 (s, br, 1H, NH, D_2O exchangeable)	288	C, 68.39, H, 6.25, N, 13.92 (C, 68.65, H, 6.44, N, 14.14)
10	**7j**	$C_{16}H_{17}N_3O_3$ (299)	184 (83)	3200 (NH) 1610 (C=O)	2.6 (s, 3H, CH_3), 2.8 (s, 3H, CH_3), 3.8 (s, 3H, OCH_3), 5.2 (s, 1H, OH), 5.7 (s, 1H, H-2), 6.9 (s, 1H, H-6), 7.2–7.4 (s, 3H, Ar–H), 8.6 (s, br, 1H, NH, D_2O exchangeable), 8.8 (s, br, 1H, NH, D_2O exchangeable)	300	C, 64.42, H, 5.84, N, 14.13 (C, 64.20, H, 5.72, N, 14.04)
11	**7k**	$C_{15}H_{15}N_3O_2$ (269)	193 (75)	3200 (NH) 1610 (C=O)	2.6 (s, 3H, CH_3), 2.8 (s, 3H, CH_3), 5.2 (s, 1H, OH), 5.7 (s, 1H, H-2), 6.9 (s, 1H, H-6), 7.2–7.4 (s, 4H, Ar–H), 8.7 (s, br, 1H, NH, D_2O exchangeable), 8.8 (s, br, 1H, NH, D_2O exchangeable)	270	C, 66.88, H, 5.55, N, 15.73 (C, 66.90, H, 5.61, N, 15.60)

Table 1 (*continued*)

S. No.	Compound code	Mol. for. (Mol. wt.)	Melting point (°C) (yield %)	IR (cm^{-1}) KBr discs	^1H NMR ($CDCl_3$/DMSO-d_6) δ	Mass *m/z* (M^+)	CHN analyses found (calcd)
12	**10a**	$C_{13}H_{13}N_3O_2$ (243)	203 (83)	3208 (NH) 1602 (C=O)	2.3 (s, 3H, CH_3), 2.6 (s, 3H, CH_3), 6.3 (s, 1H, H-6), 6.6 (s, 1H, H-2), 6.06 (d, 1H, H2-furyl), 6.36 (m, 1H, H3-furyl), 7.3 (d, 1H, H4-furyl), 8.2 (s, br, 1H, NH, D_2O exchangeable), 8.5 (s, br, 1H, NH, D_2O exchangeable)	244	C, 64.39, H, 5.25, N, 17.62 (C, 64.17, H, 5.39, N, 17.28)
13	**10b**	$C_{13}H_{13}N_3OS$ (259)	210 (85)	3208 (NH) 1602 (C=O)	2.4 (s, 3H, CH_3), 2.8 (s, 3H, CH_3), 6.3 (s, 1H, H-6), 6.6 (s, 1H, H-2), 6.06 (d, 1H, H2-thiophenyl), 6.72 (m, 1H, H3-thiphenyl), 6.71 (d, 1H, H4-thiophenyl), 8.3 (s, br, 1H, NH, D_2O exchangeable), 8.5 (s, br, 1H, NH, D_2O exchangeable)	260	C, 60.39, H, 5.25, N, 16.42 (C, 60.21, H, 5.06, N, 16.21)

[a] ^{13}C NMR data.

were obtained from Institute of Microbial Technology (IMTECH), Chandigarh. Cultures of test organisms were maintained on nutrient agar slants and were subcultured in petri dishes prior to testing. The media used was nutrient agar (procured from Himedia Laboratories, Mumbai). The minimum inhibitory concentration (MIC) was determined by the test tube dilution technique using ciprofloxacin as standard.

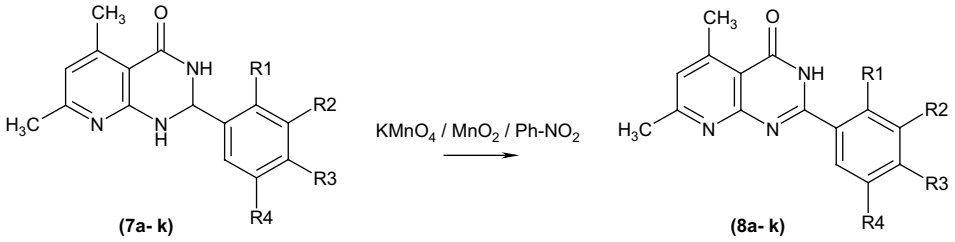
4. Results and discussion

All the synthesized compounds (**7**, **8**, **10** and **11**) were screened for their *in vitro* antibacterial activity against Gram +ve (*B. subtilis* and *S. aureus*) and Gram −ve (*E. coli* and *K. pneumoniae*) bacteria. All the dihydro compounds (**7**) showed significantly better activity over their oxidized analogues (**8**) against Gram +ve and Gram −ve bacteria. Compound **7c** showed potent activity against all species of Gram +ve and Gram −ve bacteria compared to other compounds. Enhanced activity was observed in the case of **7c** due to the presence of methoxy groups. Replacing methoxy group with methyl, hydroxyl and chloro groups resulted in lower activity. Compounds **7a** and **7i** showed better activity against Gram +ve bacteria compared to Gram −ve bacteria. Compound **7h** was more active against *S. aureus*. Compounds **7b–d**, **7f**, **7g**, **7j** and **7k** showed moderate activity against both Gram +ve and Gram −ve bacteria. Compound **7e** was the least active among the test compounds and inactive against *E. coli* (100 μg/ml concentration). Compounds **10a** and **10b** showed potent antibacterial activity against Gram +ve than Gram −ve bacteria. All the oxidized compounds (**8** and **11**) exhibited lesser antibacterial activity than dihydro analogues (**7** and **10**). Among all pyridopyrimidines, C-2 substitutions having electron releasing groups exhibited better antibacterial activity compared to substitutions having electron withdrawing groups. Compounds having more number of methoxy groups showed better activity, replacing the methoxy groups with methyl, hydroxyl and hydrogen found to be less active. Replacing substituted six-member ring with unsubstituted five-member ring at C-2 exhibited less activity. The MIC values of the compounds were compared with ciprofloxacin sample. The details of the test compounds and their activity against different microorganisms are given in Tables 4 and 5.

All the test compounds were screened for antifungal activity by using *Aspergillus niger* and *Candida albicans* and found inactive (maximum concentration of 200 μg/ml), Subsequently all the test compounds **7**, **8**, **10** and **11** were screened for their cytotoxicity *in vitro* on MCF-7 cell lines as per the method described by Moon et al. [26] and found to be non-toxic up to 150 μg/ml concentration.

5. Experimental

Melting points were recorded on Casiaa siamea (VMP-AM) melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin–Elmer FT-IR 240-C spectrophotometer using KBr discs. ^1H NMR spectra were recorded on Gemini Varian 200 MHz and 400 MHz spectrometer in $CDCl_3$ and DMSO-d_6 using TMS as internal standard. ^{13}C NMR was recorded on Bruker Avance II 400 MHz spectrometer in DMSO-d_6 using TMS as internal standard. Mass spectra



Scheme 4.

Table 2
Physical, spectral and analytical data of 2-substituted-5,7-dimethyl pyrido[2,3-*d*]pyrimidin-4(1*H*)-ones (**8** and **11**)

S. No.	Compound code	Mol. for. (Mol. wt.)	Melting point (°C) (yield %)	IR (cm^{-1}) KBr discs	^1H NMR ($CDCl_3$/DMSO-d_6) δ	Mass *m/z* (M^+)	CHN analyses found (calcd)
1	**8a**[a]	$C_{17}H_{17}N_3O_3$ (311)	254 (84)	3200 (NH) 1610 (C=O)	2.6 (s, 3H, CH_3), 2.8 (s, 3H, CH_3), 3.9 (s, 6H, OCH_3), 6.5 (s, 1H, H-6), 6.9–7.6 (d, 3H, Ar–H), 8.9 (s, br, 1H, NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6, 400 MHz): δ (ppm) = 21.5 (CH_3); 24.3 (CH_3); 55.5 (2C), 110.1, 110.4, 110.5, 112.1, 121.4, 123.5, 124.1, 148.2, 150.8, 151.6, 154.3, 163.4, 170.8	312	C, 65.49, H, 5.53, N, 13.82 (C, 65.57, H, 5.51, N, 13.50)
2	**8b**	$C_{16}H_{15}N_3O_2$ (281)	230 (82)	3200 (NH) 1602 (C=O)	2.6 (s, 3H, CH_3), 2.8 (s, 3H, CH_3), 3.9 (s, 3H, OCH_3), 6.5 (s, 1H, H-6), 6.9–7.4 (d, 4H, Ar–H), 8.8 (s, br, 1H, NH, D_2O exchangeable)	282	C, 68.42, H, 5.52, N, 14.74 (C, 68.30, H, 5.38, N, 14.94)
3	**8c**	$C_{18}H_{19}N_3O_4$ (341)	202 (92)	3206 (NH) 1610 (C=O)	2.6 (s, 3H, CH_3), 2.8 (s, 3H, CH_3), 3.9 (s, 9H, OCH_3), 6.9 (s, 1H, H-6), 7.4 (s, 2H, Ar–H), 8.8 (s, br, 1H, NH, D_2O exchangeable)	342	C, 63.46, H, 5.43, N, 12.02 (C, 63.32, H, 5.61, N, 12.31)
4	**8d**	$C_{17}H_{17}N_3O_3$ (311)	230 (86)	3200 (NH) 1610 (C=O)	2.6 (s, 3H, CH_3), 2.8 (s, 3H, CH_3), 3.9 (s, 6H, OCH_3), 6.9 (s, 1H, H-6), 7.6–7.9 (d, 3H, Ar–H), 8.9 (s, br, 1H, NH, D_2O exchangeable)	312	C, 65.49, H, 5.53, N, 13.82 (C, 65.57, H, 5.51, N, 13.50)
5	**8e**	$C_{15}H_9N_3OCl_2$ (318)	230 (60)	3210 (NH) 1600 (C=O)	2.6 (s, 3H, CH_3), 2.8 (s, 3H, CH_3), 6.9 (s, 1H, H-6), 7.2–7.4 (d, 3H, Ar–H), 8.8 (s, br, 1H, NH, D_2O exchangeable)	320	C, 56.54, H, 2.56, N, 13.73 (C, 56.61, H, 2.85, N, 13.51)
6	**8f**	$C_{17}H_{17}N_3O$ (279)	192 (70)	3208 (NH) 1620 (C=O)	2.2 (s, 6H, CH_3), 2.5 (s, 3H, CH_3), 2.8 (s, 3H, CH_3), 6.5 (s, 1H, H-6), 7.4 (s, 3H, Ar–H), 8.9 (s, br, 1H, NH, D_2O exchangeable)	280	C, 73.23, H, 6.43, N, 14.82 (C, 73.09, H, 6.13, N, 15.04)
7	**8g**	$C_{17}H_{15}N_3O_3$ (309)	260 (82)	3210 (NH) 1606 (C=O)	2.6 (s, 3H, CH_3), 2.8 (s, 3H, CH_3), 4.2 (s, 3H, OCH_3), 6.9 (s, 1H, H-6), 6.9–7.5 (d, 4H, Ar–H), 8.8 (s, br, 1H, NH, D_2O exchangeable)	310	C, 65.89, H, 5.03, N, 13.82 (C, 66.00, H, 4.89, N, 13.59)
8	**8h**	$C_{16}H_{15}N_3O_3$ (297)	250 (86)	3200 (NH) 1610 (C=O)	2.6 (s, 3H, CH_3), 2.8 (s, 3H, CH_3), 4.0 (s, 3H, OCH_3), 6.9 (s, 1H, H-6), 7.2–7.4 (d, 3H, Ar–H), 8.9 (s, br, 1H, NH, D_2O exchangeable)	298	C, 64.48, H, 5.33, N, 14.52 (C, 64.62, H, 5.09, N, 14.44)
9	**8i**	$C_{17}H_{17}N_3O_2$ (295)	252 (80)	3200 (NH) 1610 (C=O)	2.2 (s, 6H, CH_3), 2.6 (s, 3H, CH_3), 2.8 (s, 3H, CH_3), 6.9 (s, 1H, H-6), 7.2 (s, 2H, Ar–H), 8.7 (s, br, 1H, NH, D_2O exchangeable)	296	C, 69.44, H, 5.62, N, 14.04 (C, 69.12, H, 5.81, N, 14.23)
10	**8j**	$C_{16}H_{15}N_3O_3$ (297)	258 (68)	3200 (NH) 1610 (C=O)	2.6 (s, 3H, CH_3), 2.8 (s, 3H, CH_3), 3.8 (s, 3H, OCH_3), 5.2 (s, 1H, OH), 6.9 (s, 1H, H-6), 7.2–7.4 (s, 3H, Ar–H), 8.6 (s, br, 1H, NH, D_2O exchangeable)	298	C, 64.42, H, 5.01, N, 14.22 (C, 64.63, H, 5.08, N, 14.13)
11	**8k**	$C_{15}H_{13}N_3O_2$ (267)	250 (65)	3200 (NH) 1610 (C=O)	2.6 (s, 3H, CH_3), 2.8 (s, 3H, CH_3), 5.2 (s, 1H, OH), 6.9 (s, 1H, H-6), 7.2–7.4 (s, 4H, Ar–H), 8.7 (s, br, 1H, NH, D_2O exchangeable)	268	C, 67.28, H, 5.01, N, 15.83 (C, 67.40, H, 4.90, N, 15.72)
12	**11a**	$C_{13}H_{11}N_3O_2$ (241)	203 (68)	3208 (NH) 1602 (C=O)	2.3 (s, 3H, CH_3), 2.6 (s, 3H, CH_3), 6.3 (s, 1H, H-6), 6.06 (d, 1H, H2-furyl), 6.36 (m, 1H, H3-furyl), 7.3 (d, 1H, H4-furyl), 8.9 (s, br, 1H, NH, D_2O exchangeable)	242	C, 64.54, H, 4.55, N, 17.53 (C, 64.71, H, 4.60, N, 17.42)
13	**11b**	$C_{13}H_{11}N_3OS$ (257)	210 (65)	3208 (NH) 1602 (C=O)	2.4 (s, 3H, CH_3), 2.8 (s, 3H, CH_3), 6.3 (s, 1H, H-6), 6.6 (d, 1H, H2-thiophenyl), 6.72 (m, 1H, H3-thiphenyl), 6.71 (d, 1H, H4-thiophenyl), 8.9 (s, br, 1H, NH, D_2O exchangeable)	258	C, 60.39, H, 4.25, N, 16.42 (C, 60.69, H, 4.31, N, 16.34)

[a] ^{13}C NMR data.

Table 3
Percentage yields of compounds (**8**) by different oxidation methods

Compounds	Oxidation by $KMnO_4$	Oxidation by MnO_2	Oxidation by nitrobenzene
8a	48	41	84
8b	41	42	82
8c	50	46	92
8d	46	41	86
8e	39	33	60
8f	33	37	70
8g	30	33	82
8h	42	41	86
8i	40	39	80
8j	40	33	68
8k	43	30	65

(EI and CI) were recorded on a VG 7070 H instrument at 70 eV. All the reactions were monitored by thin layer chromatography (TLC) on precoated silica gel 60 F_{254} (mesh) (E Merck, Mumbai), spots were visualized under UV light (254 nm). Merck silica gel (100–200 mesh) was used for chromatography and CHN analyses were recorded on a Vario EL analyzer.

5.1. 2-Amino-4,6-dimethyl nicotinamide (5)

Ethylcyanoacetate (**1**), absolute alcohol were cooled to 0–5 °C, to this was added dry ether and maintained 0–5 °C while passing dry HCl until its weight increased. Then the reaction mass was kept aside for 24 h at 0 °C. A colorless solid precipitated out, filtered and dried under nitrogen atmosphere. Compound (**2**) was found to very unstable; all the procedures were carried out under nitrogen environment. Absolute alcohol was saturated by passing gaseous ammonia at 0–5 °C for 1 h. To this was added ethyl-β-emino-β-ethoxy acrylate hydrochloride (**2**) at once, pH was maintained at ~10 and further ammonia gas was passed at 0–5 °C for 4 h. The reaction mixture was kept aside for 5 days at room temperature, which was filtered and dried (*in vacuo*). Compound (**3**) was purified by recrystallization from ethyl acetate. Potassium hydroxide was slowly added to methanol under nitrogen atmosphere at 20–25 °C. Malonamamidine hydrochloride (**3**) was added to it at 20–25 °C to remove salt form and acetyl acetone was added to it at 25–30 °C under nitrogen environment and the reaction mixture was stirred at room temperature for 24 h. The resulted solid (KCl) was filtered off and the filtrate was concentrated by removing methanol. The viscous residue was cooled to 10 °C to get a precipitate which was filtered and dried. Compound (**5**) was purified by recrystallized from ethyl acetate. Yield 82%; m.p. 182 °C; IR (KBr) cm^{-1}: 3450, 3200, 1640 ($-CONH_2$). ^1H NMR ($CDCl_3$) δ: 2.4 (3H, s, CH_3), 2.6 (3H, s, CH_3), 4.2 (2H, s, br, $-NH_2$), 6.2 (2H, s, br, $-CONH_2$), 6.8 (1H, s, H-5). MS *m/z*: 166 (M^+). Anal. Calcd for $C_8H_{11}N_3O$: C, 58.17, H, 6.71, N, 25.44. Found: C, 58.29, H, 6.53, N, 25.31.

5.2. 2-Substituted-5,7-dimethyl dihydropyrido[2,3-d] pyrimidin-4(1H)-ones (7)

General procedure. 2-Amino-4,6-dimethyl nicotinamide (**5**) (0.00606 mol) was dissolved in glacial acetic acid (15 ml) and to it was added suitably substituted aryl aldehyde (**6a–k**) (0.00606 mol). The mixture was heated under reflux in an oil bath for 7 h. After complete conversion of starting materials (monitored by TLC) the reaction mixture was cooled, poured onto crushed ice and neutralized with aqueous ammonia to give a precipitate. It was filtered and dried (*in vacuo*). The resulted solid product was purified by column chromatography using *n*-hexane and ethyl acetate as mobile phase (95:5).

5.3. 2-Substituted-5,7-dimethyl pyrido[2,3-d]pyrimidin-4(1H)-ones (8)

General procedure. A mixture of **7** (0.5 g) and nitrobenzene (5 ml) was refluxed for 5 h. The reaction mixture was subjected to steam distillation to remove nitrobenzene and the solid obtained was filtered, washed with petroleum ether and dried.

Table 4
MIC (in μg/ml) values of 2-substituted-5,7-dimethyl dihydropyrido[2,3-*d*]pyrimidin-4(1*H*)-ones (**7** and **10**)

Compounds	Molecular formula	Microorganisms			
		Gram positive		Gram negative	
		Bacillus subtilis	*Staphylococcus aureus*	*Escherichia coli*	*Klebsiella pneumoniae*
7a	$C_{17}H_{19}N_3O_3$	3.25	3.25	6.25	12.50
7b	$C_{16}H_{17}N_3O_2$	6.25	12.50	3.25	6.25
7c	$C_{18}H_{21}N_3O_4$	3.25	1.25	3.25	1.25
7d	$C_{17}H_{19}N_3O_3$	3.25	12.50	25.00	12.50
7e	$C_{15}H_{11}N_3OCl_2$	25.00	50.00	–	100.00
7f	$C_{17}H_{19}N_3O$	6.25	12.50	12.50	25.00
7g	$C_{17}H_{17}N_3O_3$	6.25	12.50	25.00	50.00
7h	$C_{16}H_{17}N_3O_3$	12.50	1.25	50.00	50.00
7i	$C_{17}H_{19}N_3O_2$	3.25	3.25	6.25	12.50
7j	$C_{16}H_{17}N_3O_3$	25.00	25.00	50.00	25.00
7k	$C_{15}H_{15}N_3O_2$	25.00	50.00	50.00	50.00
10a	$C_{13}H_{13}N_3O_2$	3.25	12.50	25.00	12.50
10b	$C_{13}H_{13}N_3OS$	6.25	12.50	12.50	25.00
Ciprofloxacin		0.78	0.39	0.39	0.78

Table 5
MIC (in μg/ml) values of 2-substituted-5,7-dimethyl pyrido[2,3-*d*]pyrimidin-4(1*H*)-ones (**8** and **11**)

Compounds	Molecular formula	Microorganisms			
		Gram positive		Gram negative	
		Bacillus subtilis	*Staphylococcus aureus*	*Escherichia coli*	*Klebsiella pneumoniae*
8a	$C_{17}H_{17}N_3O_3$	6.25	6.25	12.50	12.50
8b	$C_{16}H_{15}N_3O_2$	25.00	12.50	12.50	25.00
8c	$C_{18}H_{19}N_3O_4$	12.50	6.25	25.00	6.25
8d	$C_{17}H_{17}N_3O_3$	12.50	25.00	25.00	50.00
8e	$C_{15}H_9N_3OCl_2$	50.00	100.00	–	–
8f	$C_{17}H_{17}N_3O$	12.50	12.50	12.50	50.00
8g	$C_{17}H_{15}N_3O_3$	25.50	25.00	25.00	50.00
8h	$C_{16}H_{115}N_3O_3$	25.00	12.50	50.00	50.00
8i	$C_{17}H_{17}N_3O_2$	12.50	6.25	6.25	12.50
8j	$C_{16}H_{15}N_3O_3$	50.00	50.00	50.00	50.00
8k	$C_{15}H_{13}N_3O_2$	50.00	50.00	50.00	100.00
11a	$C_{13}H_{11}N_3O_2$	12.50	25.00	25.00	50.00
11b	$C_{13}H_{11}N_3OS$	12.50	25.00	12.50	50.00
Ciprofloxacin		0.78	0.39	0.39	0.78

5.4. Antibacterial assays (in vitro)

All the test compounds was assayed *in vitro* for antibacterial activity against different strains of Gram −ve [*E. coli* (MTCC 722), and *K. pneumoniae* (MTCC 109)] and Gram +ve [*B. subtilis* (MTCC 441) and *S. aureus* (MTCC 96)] bacteria using standard protocol [27]. The minimum inhibitory concentration (MIC) was determined by the test tube dilution technique using ciprofloxacin as standard. The stock solution (1 mg/ml) of test compounds was prepared in DMSO. The stock solution was sterilized by passing through a 0.2 mm polycarbonate sterile membrane (Nuclepore) filter. Further the serial dilution of test compounds was carried out and the following concentrations were used: 100, 50, 25, 12.5, 6.25, 3.25, 1.25 μg/ml. Test compounds at various concentrations were added to culture medium in a sterilized borosilicate tube and different bacterial strains were inoculated at 106 bacilli/ml concentration. The tubes were incubated at 37 °C for 24 h and then examined for the presence or absence of growth of the test organisms. All experiments were performed in triplicate. The MIC values were obtained from the lowest concentration of the test compound where the tubes remained clear, indicated that the bacterial growth was completely inhibited at this concentration.

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