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Installation of biologically active pyrimidine moiety into pyridopyrimidine framework and evaluation of their antibacterial activities

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Pyrido[2,3-*d*]pyrimidines **3** have been synthesized starting from 5-benzylidene barbiturate and maloninitrile in presence of ammonium acetate. Installation of pharmacologically active pyrimidine moiety into pyridopyrimidine framework **3** has been accomplished by condensation of **3** with formic acid, acetic acid, urea, thiourea, formamide and hydrazine hydrate. The antibacterial activities of the synthesized compounds have been evaluated. Good to moderate activities of the tested compounds have been observed.

Keywords: Pyrimidine, pyrido[2,3-*d*]pyrimidine, 5-arylidene-barbituric acids, maloninitrile

Pyridopyrimidines are annelated uracils that have attracted considerable interest in recent years. The pyrido[2,3-*d*]pyrimidine ring system is present in a number of biologically active organic compounds which includes antitumour[1], antibacterial[2], antiviral[3], antioxidant[4], antifungal[5], hepatoprotective[6] and anticonvulsant agents[2]. It also has antipyretic[7], analgesic[8] and CNS depressant activity[9]. More specifically, pyrido [2,3-*d*] pyrimidines were considered as inhibitors of *Pneumocystis carinii* (pc), *Toxoplasma gondii* (tg), of tumour cell lines in culture[10] and some have anticytokinin activity[11]. The activity is mainly due to inhibition of dihydrofolate reductase (DHFR)[12,13].

In view of significant biological contribution of pyridopyrimidine heterocycles and in continuation of our work on the synthesis of biologically active heterocycles[14], herein is reported the installation of another pyrimidine moiety into pyridopyrimidine framework possessing antibacterial activity with expectations of an enhancement of biological properties of target molecules using barbiturates as synthetic precursors.

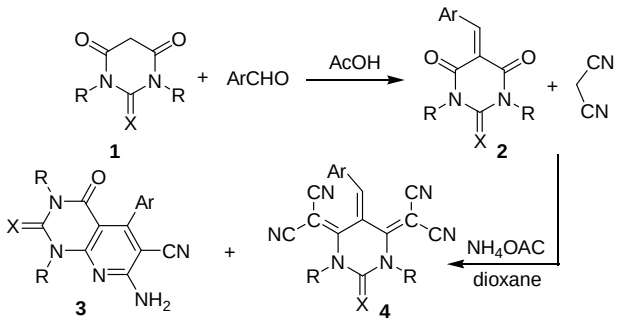
Results and Discussion

5-Arylidene-barbituric acids **2** was synthesized by condensation of appropriate barbiturates **3** with equimolar amount of various arylaldehydes in acetic acid as previously reported Scheme I (Ref 15). The one-pot reaction of 5-arylidene-1,3-diphenyl thiobarbituric acids **2a-f**, malononitrile and excess of ammonium acetate in dioxane produced pyrido-pyrimidine-6-carbonitrile derivatives **3a-f**.

The effect of substituents of aryl system in arylidene derivatives **2a-f** in the formation of **3a-f** has also been studied. The reaction of **2b** (X=S, R=Ph, Ar = *p*-$OMeC_6H_4$), and **2c** (R=Ph; *p*-$N(Me)_2C_6H_4$) with malononitrile and ammonium acetate afforded the mixture of products **3b,c** isolated from water and the corresponding **4a,b** formed in the reaction set in 51%, 42% and 20%, 35% yield, respectively.

The possible explanation of the reaction mechanism for the product **3a-f** is supposed to occur through non-isolable Michael adduct formed by refluxing the mixture of arylidene **2a-f** and malononitrile under the above said reaction conditions and subsequent *in situ* oxidation of the intermediate. The regioselective reaction of arylidene derivative with malononitrile is monitored by *p*-substituted benzaldehyde to form the products **3a-f** and **4a,b**. In fact, *p*-NO_2, *p*-Cl and *p*-Br substituents at the phenyl ring increases the reactivity at β-carbon atom of α,β-unsaturated carbonyl scaffold of arylidene derivatives due to –M effect. On the contrary, the

selectivity of reaction of malononitrile increases at carbonyl carbon of the arylidene derivatives **2b,c** caused by +M effect due to *p*-OMe and *p*-Me_2N substituents at phenyl ring (Scheme I). Subsequently, another set of 5-arylidene barbituric acid **2g-j** (R = H, X = O) was examined as the replacement of 5-arylidene 1,3-diphenyl thiobarbituric acid **2a-f** acid in the reaction with malononitrile and ammonium acetate Scheme I. Surprisingly, under the reaction conditions described above, a mixture of **4c-f** as major product and **3g-j** being the minor were generated. Here, in case of 5-arylidene barbituric acid (R = H, X = O) the comparative increase in the reactivity of carbonyl function at 4 and 6 positions of the arylidene intermediate **2** (X = O, R = H) renders significant contribution in the formation of the major product **4** due to the presence of uriedo scaffold. This is still a puzzle to us, and further efforts are underway to clarify this problem. The IR spectrum of **2a** (R = Ph; X = S) shows bathochromic shift of two carbonyl stretching frequencies from 1728 and 1709 cm^{-1} in **1** to 1712 and 1683 cm^{-1} suggesting the formation of exocyclic double bond at position-5 of thiobarbiturate scaffold. The characteristic peaks in the region 3176-3446 cm^{-1} and 2140-2194 cm^{-1} corresponding to -NH and −C≡N stretching band, respectively in the IR spectrum of **3a-f**, clearly demonstrate the formation of pyridopyrimidine derivative **3a-f**. Disappearance of one of carbonyl band at 1712 cm^{-1} in **2** and leaving the other carbonyl group unaffected suggests



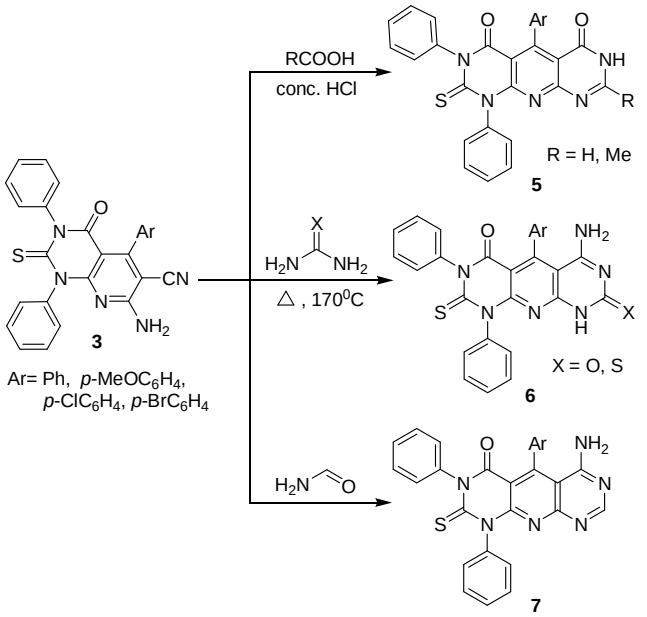
3a: X= S; R= Ph, Ar= Ph
3b: X= S; R= Ph, Ar= *p*-$OMeC_6H_4$
3c: X= S; R= Ph, Ar= *p*-$N(Me)_2C_6H_4$
3d: X= S; R= Ph, Ar= *p*-ClC_6H_4
3e: X= S; R= Ph, Ar= *p*-$Br_2C_6H_4$
3f: X= S; R= Ph, Ar= *p*-$NO_2C_6H_4$
3g: X= O; R= H, Ar= Ph
3h: X= O; R= H, Ar= *p*-$OMeC_6H_4$
3i: X= O; R= H, Ar= *p*-ClC_6H_4
3j : X= O; R= H, Ar= *p*-BrC_6H_4

4a: X= S; R= Ph, Ar= *p*-$OMeC_6H_4$
4b: X= S; R= Ph, Ar= *p*-$N(Me)_2C_6H_4$
4c: X= O; R= H, Ar= Ph
4d: X= O; R= H, Ar= *p*-$OMeC_6H_4$
4e: X= O; R= H, Ar= *p*-ClC_6H_4
4f : X= O; R= H, Ar= *p*-BrC_6H_4

Scheme I — Synthesis of pyridopyrimidine-6-carbonitrile derivative **3** and 2-[6-dicyanomethylene-5-arylidene-pyrimidin-4-ylidene]-malononitrile **4**

unambiguously the participation of more active carbonyl function in the process of cyclocondensation followed by *in situ* oxidation. The appearance of two carbonyl stretching frequencies at 1688-1691 and 1646-1649 cm^{-1} along with the presence of nitrile peak at 2129-2136 cm^{-1} in the IR spectrum, suggests the formation of the pyridopyrimidine derivatives **3g-j**. In the ^{1}H NMR spectrum of compounds **3a-j**, a broad singlet at δ 4.43-4.96 exchangeable with D_2O and a complex multiplet resonating at δ 6.57-7.91 corresponding to NH_2 and phenyl protons, respectively confirms the formation of **3a-j**. ^{13}C NMR spectrum of compound **3a-f** and **3g-j** showed signals at δ 173.15-174.66 and 155.21-157.32, 159.10-159.23 due to carbonyl groups, respectively. In addition, elemental analyses are consistent with the structures proposed for the compounds **3a-j**. The formation of dicyano pyrimido derivatives **4a,b** was ensured by the disappearance of both carbonyl bands of **2b,c** and appearance of nitrile peak at 2221-2222 cm^{-1} in the IR spectrum while the dicyano pyrimido derivatives **4c-f** displayed absorption bands at 2222-2224 cm^{-1} and 1672-1676 cm^{-1} corresponding to nitrile and carbonyl groups, respectively. In the ^{1}H NMR spectrum of **4a-f** a sharp singlet emerged at δ 8.03-8.39 for benzylidene proton.

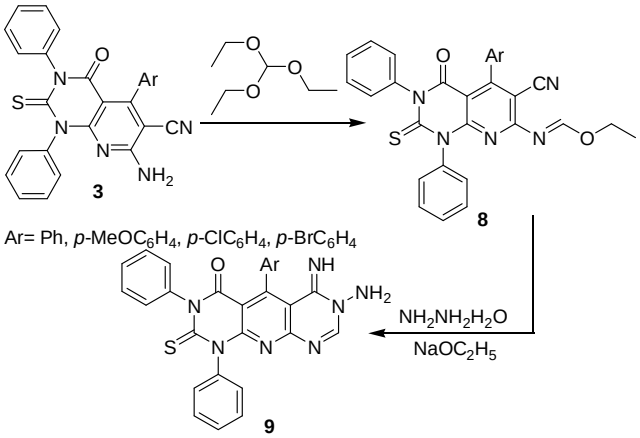
In continuation of our work on the synthesis[14] of new fused heterocyclic systems having potential biological activites, we have installed another pyrimidine moiety into pyridopyrimidine framework **3a,b,d-f**. The pyrido [2,3-*d*]pyrimidine-6-carbonitrile **3a,b,d-f** being a β-enaminonitrile derivative, pyrimidine ring was annulated with **3a,b,d-f** in presence of formic acid/acetic acid and catalytic amount of conc. HCl *via* partial hydrolysis of nitrile group in **3a,b,d-f** to amide group followed by dehydrative cyclisation to form pyrimido pyrido pyrimidine derivative **5** in good yield (Scheme II). Disappearance of CN stretching, and appearance of second carbonyl stretching in the lower frequency region at 1645-1647 cm^{-1} assignable to cyclic amide function in the IR spectrum of **5a-h** are in favour of the proposed structure **5a-h**. In the ^{1}H NMR spectrum of **5a-d** and **5e-h**, the methine and methyl protons of pyrimidin-4(3*H*)-one ring appeared as singlet at δ 8.53-8.61 and δ 1.80-1.89, respectively. The ^{1}H NMR spectrum of **5a-h** also showed a broad singlet at δ 12.05-12.29 exchangeable with D_2O for NH proton. ^{13}C NMR spectrum of compound **5a-h** showed signals at δ 160.80-160.98 and δ 159.65-160.21 due to carbonyl group of PhNC=O and -NHC=O,



Scheme II — Synthesis of pyrimido pyrido pyrimidine derivative **5-7**

respectively. These signals clearly demonstrate the formation of pyrimido pyrido pyrimidine derivative **5a-h**. The analytical data also supports the formation of the product **5a-h**.

Pyrimido pyrido pyrimidine **6** was constructed by condensation of equimolar pyridopyrimidine derivative **3a**, **b**, **d-f** and urea/ or thiourea on a sand bath at 170°C in good yield *via* deaminative cyclisation. The appearance of absorption bands at 3285-3301 cm^{-1} and 3185-3198 cm^{-1} corresponding to NH_2 and NH stretchings in the IR spectrum of **6a-h** along with absorption bands for carbonyl groups at 1664-1671 for **6a-h** and 1645-1646 cm^{-1} for **6e-h** ensures the formation of pyrimido pyrido pyrimidine derivative **6a-h**. The ^1H NMR spectrum of **6a-h** exhibited two distinct singlets at δ 7.85-8.02 and δ 8.55-8.78 corresponding to two protons of NH_2 and one proton of NH groups, respectively. Moreover, in the ^{13}C NMR spectra of compounds **6a-h**, the –C=S and –C=O signals were observed at δ 180.07-181.09 and δ 152.95-153.64, respectively. Furthermore, the elemental analyses are compatible with the structure **6a-h**. The reaction of formamide with **3a,b,d-f** under reflux generated pyrimido pyrido pyrimidine derivatives **7**. The IR spectrum of compounds **7a-d** displayed absorption bands in the region 1668-1670 cm^{-1} and 3188-3371 cm^{-1} corresponding to carbonyl and NH_2 groups, respectively. The ^1H NMR spectrum of **7a-d** showed a singlet at δ 9.87-9.98 for CH proton of pyrimidine ring. A multiplet for aromatic protons



Scheme III — Synthesis of pyrimido pyrido pyrimidine derivative **9**

appeared at δ 6.60-7.56 and signals due to NH_2 protons were found to be merged with aromatic protons suggesting the formation of **7a-d**.

The condensation of equimolar mixture of **3a**, **b**, **d-f** and triethylorthoformate on an oil bath produced formimidic acid ethyl ester derivative **8** which on subsequent treatment with hydrazine hydrate in refluxing ethanol in presence of NaOEt as catalyst underwent cyclisation to form pyrimido pyrido pyrimidine derivatives **9** (Scheme III). The formation of **8** is ensured on the basis of analytical and IR spectral data. Disappearance of NH stretching in the IR spectrum of **8a-d**, and appearance of a characteristic sharp absorption band at 1612-1617 cm^{-1} indicate the formation of azomethine group in the product. The retention of nitrile band at 2214-2222 cm^{-1} in the IR region **s**uggests the non-interfering of nitrile function during the reaction while that of **9a-d** revealed the absence of the nitrile group. The ^1H NMR spectrum of **8a-d** displayed a quartet at δ 4.26-4.30 for CH_2 protons and a triplet at δ 1.29-1.33 corresponding to methyl protons along with a singlet at δ 7.67-7.78 for azomethine proton suggesting the formation of ester derivative **8a-d**. The signals for the NH_2 and NH protons appearing as distinct broad singlets at δ 12.39-12.60 and δ 12.89-12.97 along with a singlet at δ 8.56-8.78 corresponding to pyrimidine proton confirm the formation of pyrimido pyrido pyrimidine derivative **9a-d**. Further, C=NH in ^{13}C NMR spectrum of **9a-d** showed peak at δ 161.92-162.89.

Antibacterial activity

The antibacterial study of the synthesized compounds was carried out using disc diffusion and

broth dilution method[16,17]. The synthesized compounds were screened for their antibacterial activity *in vitro* against Gram negative bacterium (GNB) *Escherichia coli* isolated from local pond water of Sambalpur University Campus, Odisha, India and Gram positive bacterium (GPB) *Staphylococcus aureus* isolated from drug-vial contaminants, were identified in Medical Microbiology Laboratory, School of Life Science, Sambalpur University by following routine diagnostic microbiological tests. Activity of each compound was compared with Penicilin-G, Erythromycin, Ampicilin, Cephalothin, Clindamycin, Co-Trimoxazole, and Kanamycin as standards against Gram positive and Gram negative strains respectively. The minimum inhibitory concentrations (MIC) were determined by broth dilution technique. To the nutrient broth, the overnight bacterial cultures diluted at a ratio of 1:100 counting 10^3 CFU/mL (colony forming unit) was added and graded concentrations (0.2-500 μg/mL) of the synthesized compounds in dimethyl sulfoxide were added. The cultures were incubated for 12-14 h at 37°C. Visible turbidity in the MIC tubes was taken as the parameter for determination of minimum inhibitory concentration (MIC). The lowest concentration (highest dilution) of the drug required to arrest the growth of bacteria was determined as MIC (μg/mL).A number of antimicrobial discs are placed on the agar for the sole purpose of producing zones of inhibition in the bacterial lawn. Agar media were poured into each Petri dish. Excess of suspension was decanted and placed in incubator at 37°C for 1 h to dry the plates[17]. The disc measuring 5 mm in diameter were prepared from Whatman no.1 filter paper and sterilized by dry heat at 140°C for 1 h. The sterile discs were placed in nutrient agar medium and 50 μL of minimum inhibitory concentration of the test compounds in DMSO were delivered into each labeled disc. Running control with pure DMSO as solvent, has shown no effect. The discs were prepared in triplicate in each Petri dish and incubated for 48 h at 37°C. Antibacterial activity was determined by measuring the diameter of inhibition zone. Activity of each compound was compared with Penicilin-G (10 μg), Erythromycin (10 μg), Ampicilin (25 μg), Cephalothin (30 μg), Clindamycin (2 μg), Co-Trimoxazole (25 μg), and Kanamycin (5 μg) as standard. The antibacterial activity was classified on the basis of diameter of inhibition zone such as highly active (>21 mm), moderately active (15–21 mm), least active (12–15 mm) and less than 12 mm was taken as inactive. The zones of inhibition of the standard drugs and the synthesized compounds is summarized in **Table** I. The investigation of preliminary antibacterial screening data (Table I) revealed that most of the tested compounds showed moderate to good bacterial inhibition. Compounds **5c**, **5f**, **5g**, **6f**, **7b**, **8b**, and **9b** exhibited good antibacterial activity against *Staphylococcus spp.* and *E. coli* strains. Compound **5f**, **7b**, **8a**, **8b**, and **9b** showed good activity against both *S. aureus* and *E. coli* bacterial strains whereas compounds **5c**, and **5g** illustrated good activity against *E. coli* bacterial strain. It is interesting to note that installation of another pyrimidine moiety into pyridopyrimidine framework **3** increases the antibacterial activity. Enhanced activity has been observed in cases of compounds having *p*-OMe and *p*-Cl substituents in the phenyl rings.

Experimental Section

Melting points were taken in open capillaries using sulfuric acid bath and are uncorrected. IR spectra were recorded with a Shimadzu FT-IR Prestige-21 spectrophotometer in potassium bromide (KBr) using diffuse reflectance system (DRS) technique. NMR spectra were recorded on a VARIAN 400 MHz (400 MHz for ^{1}H, 100 MHz for ^{13}C) NMR spectrometer in dimethyl sulfoxide-d_6 (DMSO), unless otherwise stated. Chemical shifts are expressed in parts per million downfield from tetramethylsilane as an internal standard. Data are given in the following order: δ value, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad), number of protons, coupling constants *J* is given in Hertz. Elemental analyses were performed by Flash 2000 CHN elemental analyzer, expressed in % and were in agreement with the calculated values within ±0.4%. All the reagents and solvents used were of the best grade available and were used without further purification.

General procedure for the synthesis of pyrido[2,3-*d*]pyrimidine-6-carbonitrile derivatives 3a-j and (pyrimidin-4-ylidene)-malononitrile derivatives, 4a-f

A mixture of the 5-benzylidenepyrimidine **2** (10 mmol), malononitrile (10 mmol) and ammonium acetate (90 mmol) in dioxane (60 mL) was refluxed with stirring for 6 h. The reaction mixture was allowed to cool to RT and the solution was poured onto crushed ice. The precipitated product thus formed was filtered, dried and recrystallised from ethanol to afford the desired product.

Table I — Antibacterial activity of synthesized compounds

Compd	Ar	R	X	Zone of Inhibition in mm (MIC in μg/mL)	
				Gram Positive Bacteria[a]	Gram Negative Bacteria[a]
				Sa	*Ec*
3a	Ph	–	S	16(26.5)	18(30.1)
3b	*p*-$OMeC_6H_4$	–	S	19(28.5)	20(29.5)
3c	*p*-$N(Me)_2C_6H_4$	–	S	17(25.4)	20(26.4)
3d	*p*-ClC_6H_4	–	S	19(26.4)	20(25.4)
3e	*p*-BrC_6H_4	–	S	14(27)	16(27.4)
3f	*p*-$NO_2C_6H_4$	–	S	16(27.1)	18(27.7)
3g	Ph	–	O	14(41)	13(32)
3h	*p*-$OMeC_6H_4$	–	O	18(32.2)	16(33.1)
3i	*p*-ClC_6H_4	–	O	16(34.2)	16(36.4)
3j	*p*-BrC_6H_4	–	O	13(35.6)	14(34.2)
5a	Ph	H	–	18(23.3)	19(25.2)
5b	*p*-$OMeC_6H_4$	H	–	19(24.5)	20(25.4)
5c	*p*-ClC_6H_4	H	–	20(23)	22(24.1)
5d	*p*-BrC_6H_4	H	–	17(26.5)	16(26.2)
5e	Ph	Me	–	19(24)	20(26.3)
5f	*p*-$OMeC_6H_4$	Me	–	21(22.9)	21(22.1)
5g	*p*-ClC_6H_4	Me	–	18(24.6)	22(25.4)
5h	*p*-BrC_6H_4	Me	–	14(27)	16(27.4)
6a	Ph	–	O	17(24.2)	18(25.1)
6b	*p*-$OMeC_6H_4$	–	O	18(25.3)	19(27.2)
6c	*p*-ClC_6H_4	–	O	17(24.3)	19(23)
6d	*p*-BrC_6H_4	–	O	16(26.1)	18(26.3)
6e	Ph	–	S	19(23.9)	19(25.3)
6f	*p*-$OMeC_6H_4$	–	S	19(25.1)	21(26.2)
6g	*p*-ClC_6H_4	–	S	18(24)	20(23.2)
6h	*p*-BrC_6H_4	–	S	17(24.6)	19(25.2)
7a	Ph	–	–	18(23)	19(25)
7b	*p*-$OMeC_6H_4$	–	–	20 (25.6)	21(26.5)
7c	*p*-ClC_6H_4	–	–	16(24.2)	20(23.5)
7d	*p*-BrC_6H_4	–	–	15(24.8)	19(25.9)
8a	Ph	–	–	20(22.1)	19(24.9)
8b	*p*-$OMeC_6H_4$	–	–	21(25)	20(25.9)
8c	*p*-ClC_6H_4	–	–	19(24.2)	18(23.6)
8d	*p*-BrC_6H_4	–	–	16(24.2)	17(25.6)
9a	Ph	–	–	19(23.7)	17(25.1)
9b	*p*-$OMeC_6H_4$	–	–	21(24.5)	20(25.1)
9c	*p*-ClC_6H_4	–	–	17(22.7)	19(23)
9d	*p*-BrC_6H_4	–	–	18(24.9)	17(26.1)
Penicilin-G[b]	–	–	–	0	0
Erythromycin[b]	–	–	–	22	20
Ampicilin[b]	–	–	–	0	0
Cephalothin[b]	–	–	–	0	0
Clindamycin[b]	–	–	–	26	12
Co-Trimoxazole[b]	–	–	–	0	21
Kanamycin[b]	–	–	–	12	15.5

In case when X, R, Ar = S, Ph, *p*-$OMeC_6H_4$; S, Ph, *p*-$N(Me)_2C_6H_4$; O, H, Ph; O, H, *p*-$OMeC_6H_4$; O, H, *p*-ClC_6H_4; O, H, *p*-BrC_6H_4, the solid formed in the reaction set during reflux was filtered, dried and recrystallised from DMF to give the respective (pyrimidin-4-ylidene)-malononitrile derivatives **4a-f**. Thereafter, the filtrate was poured onto crushed ice. The solid thus formed was filtered, dried and recrystallised from ethanol to yield the respective pyrido[2,3-*d*]pyrimidine derivatives **3b,c** and **3g-j**.

7-Amino-4-oxo-1,3,5-triphenyl-2-thioxo-1,2,3,4-tetrahydropyrido [2,3-d]pyrimidine-6-carbonitrile, 3a: Yield 80%. m.p.150°C. IR (KBr): 3429, 3331, 3176 (-NH_2), 2196 (C≡N), 1683 cm^{-1} (C=O); ^1H NMR (DMSO-d_6, 400 MHz): δ_H 4.43 (br, 2H, -NH_2, D_2O exchangeable), 6.96-7.61 (m, 15H, PhH); ^{13}C NMR (DMSO-d_6, 400 MHz,): δ_C 79.79, 110.36, 116.39, 124.82, 125.21, 125.9, 126.54, 127.92, 128.18, 128.49, 129.32, 129.79, 133.76, 134.43, 135.02, 158.95, 159.69, 160.95, 167.29, 173.15. Anal. Calcd for $C_{26}H_{17}N_5OS$: C, 69.80; H, 3.80; N, 15.66. Found: C, 69.54; H, 3.51; N, 15.36%.

2-[6-Dicyanomethylene-5-(4-methoxy-benzylidene)-1,3-diphenyl-2-thioxo-tetrahydro-pyrimidin-4- ylidene]-malononitrile, 4a: Yield 20%. m.p.150°C. IR (KBr): 2222 cm^{-1} (C≡N); ^1H NMR (DMSO-d_6, 400 MHz): δ_H 3.88 (s, 3H, Me), 6.90-7.10 (m, 4H, PhH), 7.17-7.20 (m, 4H, PhH), 7.41-7.44 (m, 4H, PhH), 7.95-7.98 (m, 2H, PhH), 8.39 (s, 1H, C=CH); ^{13}C NMR (DMSO-d_6, 400 MHz,): δ_C 55.19, 55.85, 76.78, 113.85, 114.74, 115.14, 124.05, 126.45, 127.72, 129.87, 133.30, 160.39, 161, 164.28, 173. Anal. Calcd for $C_{30}H_{18}N_6OS$: C, 70.59; H, 3.53; N, 16.47. Found: C, 70.27; H, 3.25; N, 16.16%.

1,3,5-Triphenyl-2-thioxo-1,2,3,4-tetrahydro-pyrimido [4″,5″:2′,3′]pyrido[5,6-b]pyrimidine-4,6(7*H*)-dione, 5a

A mixture of compound **3a** (1 mmol), formic acid (10 mL) and a catalytic amount of concentrated hydrochloric acid was refluxed at 160°C for 6 h. The reaction mixture was allowed to cool to RT and poured into ice cold water. The solid thus formed was filtered, dried and recrystallised from DMF to give the respective pyrimido pyrido pyrimidine derivative **5a**. Yield 75%. m.p.220°C. IR (KBr): 3196 (NH), 1689, 1645 cm^{-1} (C=O); ^1H NMR (DMSO-d_6, 400 MHz): δ_H 6.79-7.90 (m, 15H, PhH), 8.53 (s, 1H, C8-H), 12.25 (br, 1H, -NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 400 MHz,): δ_C 99.21, 107.42, 121.32, 123.99, 124.64, 126.55, 127.36, 128.95, 129.12, 129.38, 132.81, 136.85, 137.96, 145.45, 149.59, 151.21, 160.03, 160.98, 162.22, 177.03. Anal. Calcd for $C_{27}H_{17}N_5O_2S$: C, 68.21; H, 3.58; N, 14.74. Found: C, 67.96; H, 3.32; N, 14.42.

6-Amino-1,3,5-triphenyl-2-thioxo-1,2,3,4-tetrahydro-pyrimido[4″,5″:2′,3′]pyrido[5,6-b]pyrimidine-4,8(9*H*)-dione, 6a

A mixture of compound **3a** (1 mmol), and urea (1 mmol) was heated at 170°C in a test tube on a sand-bath for 4 h. The mixture was allowed to cool to RT and to it DMF (20 mL) was added. The solution was poured into ice cold water and the solid thus formed was filtered, dried and recrystallised from DMF to give the respective pyrimido pyrido pyrimidine derivative **6a**. Yield 72%. m.p.245°C. IR (KBr): 3298 (-NH_2), 3194 (NH), 1664, 1645 cm^{-1} (C=O); ^1H NMR (DMSO-d_6, 400 MHz): δ_H 6.94-7.62 (m, 15H, PhH), 7.95 (s, 2H, -NH_2, D_2O exchangeable), 8.67 (br, 1H, -NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 400 MHz,): δ_C 90.12, 98.56, 122.16, 124.32, 124.75, 126.51, 127.33, 128.29, 129.04, 129.52, 129.86, 133.02, 136.58, 138.05, 143.12, 151.34, 153.52, 156.22, 160.87, 163.33, 177. Anal. Calcd for $C_{27}H_{18}N_6O_2S$: C, 66.12; H, 3.67; N, 17.14. Found: C, 65.84; H, 3.39; N, 16.85%.

6-Amino-1,3,5-triphenyl-2-thioxo-1,2,3,4-tetrahydro-pyrimido[4″,5″:2′,3′]pyrido[5,6-b]pyrimidine-4-one, 7a

A mixture of compound **3a** (1 mmol), formamide (5 mL), and formic acid (2 mL) was refluxed in DMF (50 mL) for 6 h. The reaction mixture was allowed to cool to RT, poured into water and neutralized with ammonia solution. The solid thus formed was filtered, dried and recrystallised from DMF to give the respective pyrimido pyrido pyrimidine derivative **7a**. Yield 70%. m.p.185°C. IR (KBr): 3361, 3309, 3188 (-NH_2), 1668 cm^{-1} (C=O); ^1H NMR (DMSO-d_6, 400 MHz): δ_H 7.00-7.56 (m, 17H, PhH and -NH_2), 9.96 (s, 1H, C8-H); ^{13}C NMR (DMSO-d_6, 400 MHz,): δ_C 98.05, 102.34, 121.30, 124.06, 124.60, 126.58, 127.34, 128.91, 129.09, 129.27, 132.75, 136.78, 138, 151.20, 155.23, 156.06, 156.99, 157.34, 160.98, 162.76, 177. Anal. Calcd for $C_{27}H_{18}N_6OS$: C, 68.35; H, 3.80; N, 17.72. Found: C, 68.07; H, 3.53; N, 17.43%.

N-(6-Cyano-4-oxo-1,3,5-triphenyl-2-thioxo-1,2,3,4-tetrahydro-pyrido[2,3-d]pyrimidin-7-yl)-formimidic acid ethyl ester, 8a

A mixture of compound **3a** (2 mmol) and triethyl orthoformate (16 mmol) was stirred under reflux in

acetic anhydride (20 mL) for 6 h. The reaction mixture was allowed to cool to RT, poured into water and neutralized with ammonia solution. The solid thus formed was filtered, dried and recrystallised from ethanol to give the respective formimidic acid ethyl ester derivative **8a**. Yield 85%. m.p.145°C. IR (KBr): 2214 (C≡N), 1683 (C=O), 1612 cm^{-1} (C=N); ^{1}H NMR (DMSO-d_6, 400 MHz): δ_H 1.29 (t, 3H, J = 7.2 Hz, Me), 4.30 (q, 2H, J = 7.2 Hz, CH_2), 7.07-7.64 (m, 15H, PhH), 7.78 (s, 1H, HC=N); ^{13}C NMR (DMSO-d_6, 400 MHz,): δ_C 21.03, 62.10, 88.32, 112.54, 117, 124.84, 125.13, 125.89, 126.55, 127.25, 27.97, 128.52, 129.11, 129.80, 132.15, 135.21, 136.29, 156.04, 157.12, 159.45, 160.85, 169.76, 175.21. Anal. Calcd for $C_{29}H_{21}N_5O_2S$: C, 69.18; H, 4.17; N, 13.92. Found: C, 68.88; H, 3.91; N, 13.60%.

7-Amino-6-imino-1,3,5-triphenyl-2-thioxo-1,2,3,4-tetrahydro-pyrimido[4″,5″:2′,3′]pyrido-[5,6-b]pyrimidine-4-one, 9a

A mixture of compound **3a** (1 mmol) and hydrazine hydrate (99%, 10 mmol) was refluxed for 3 h in ethanol (20 mL) in presence of sodium ethoxide [prepared by dissolving sodium metal (10 mmol, 0.23 g) in 20 mL absolute ethanol]. The reaction mixture was allowed to cool to RT, poured into water and neutralized with 2N HCl. The solid thus formed was filtered, dried and recrystallised from ethanol to give the respective pyrimido pyrido pyrimidine derivative **9a**. Yield 73%. m.p.160°C. IR (KBr): 3435, 3317 (-NH_2), 3197 (-NH), 1689 cm^{-1} (C=O); ^{1}H NMR (DMSO-d_6, 400 MHz): δ_H 7.22-8.04 (m, 15H, PhH), 8.78 (s, 1H, C8-H), 12.60 (br, 2H, -NH_2, D_2O exchangeable), 12.89 (br, 1H, -NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 400 MHz,): δ_C 96.7, 103.12, 121.09, 123.99, 124.38, 124.87, 126.76, 127.55, 128.23, 129.12, 129.34, 129.56, 131.77, 136.07, 137.98, 146.15, 150.03, 160.12, 162.43, 164.01, 177.12. Anal. Calcd for $C_{27}H_{19}N_7OS$: C, 66.26; H, 3.88; N, 20.04. Found: C, 66.02; H, 3.60; N, 19.73%.

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References

1 (a) Piper J R, Montgomery J A & Sirotnak F M, *US Patent* 4725687 (1989); *Chem Abstr*, 109 (1989) 129064x; (b) Piper J R, Montgomery J A, Sirotnak F J & DeGraw I, *US Patent* 5077404 (1991); *Chem Abstr*, 116 (1992) 128965z.

2 Ram V J, Berghe D A V & Vlietinck A J, *J Heterocycl Chem*, 28 (1988) 217.

3 Tenser R B, Gaydos A & Hay K A, *Antimicrob Agents Chemother*, 45 (2001) 3657.

4 de la Cruz J P, Carrasco T, Ortega G & de la Cuesta F S, *Lipids*, 27 (1992) 192.

5 Nizamuddin, Mishra M, Srivastava M K & Khan M H, *Indian J Chem*, 40B (2001) 49.

6 Ram V J, Goel A, Sarkhel S & Maulik P R, *Bioorg Med Chem*, 10 (2002) 1275.

7 Piper J R, McCalab G S, Montgomery J A, Kisliuk R L, Gaumont Y & Sirotnak F M, *J Med Chem*, 29 (1986) 1080.

8 Robins R K & Hitchings G H, *J Am Chem Soc*, 80 (1958) 3449.

9 Madkour H M F, Salem M A I, Abdel-Rahman T M & Azab M E, *Heterocycles*, 38 (1994) 57.

10 Lowe J A, *Austrian Patent* 388378 (1989); *Chem Abstr*, 112 (1990) 21008.

11 Kouichiro G, Kurimoto Y & Kitamura N, *Eur Patent Appl* 243311 (1987); *Chem Abstr*, 108 (1988) 75422.

12 Gangjee A, Adair O O & Queener S F, *J Med Chem*, 46 (2003) 5074.

13 Iwamura H, Murakami S, Koshimizu K & Matsubara S, *J Med Chem*, 28 (1985) 577.

14 (a) Behera A K, Behera R K, Pradhan R, Pati A & Patra M, *Indian J Heterocycl Chem*, 16 (2006) 167; (b) Behera R K, Behera A K, Pradhan R & Patra M, *Indian J Chem*, 45B (2006) 933; (c) Behera R K, Behera A K, Pradhan R, Pati A & Patra M, *Synth Commun*, 36 (2006) 3729; (d) Behera R K, Behera A K, Pradhan R, Pati A & Patra M, *J Chem Environ*, 10 (2006) 18; (e) Behera R K, Behera A K, Pradhan R, Pati A & Patra M, *Phosphorus, Sulfur and Silicon*, 184 (2009) 753; (f) Behera R K, Pati A, Patra M & Behera A K, *Phosphorus, Sulfur and Silicon*, 184 (2009) 2827.

15 Das P K, Behera G B, Sahay A K & Mishra B K, *Indian J Chem*, 24B (1985) 437.

16 Pattnaik S D, Padhan K & Jana G K, *J Pharm Res*, 3 (2010) 414.

17 Gillespie S H, *Medical Microbiology-Illustrated* (Butterworth Heinemann Ltd., Oxford, UK), 234 (1994).