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Dihydropyrido[2,3-*d*]pyrimidines as a new class of antileishmanial agents

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Abstract—A series of dihydropyrido[2,3-*d*]pyrimidines have been synthesized and screened for its in vitro antileishmanial activity profile in promastigote and amastigote models. Compounds **2a**–**2l** have shown 83–100% inhibition against promastigotes and 79–100% inhibition against amastigotes at a concentration of 50 μg/mL.

1. Introduction

Leishmaniasis is caused by different species belonging to the genus *Leishmania*, a protozoan which is transmitted to humans by the bite of an insect vector, phlebotomine sandfly. Leishmaniasis has an overwhelming impact on global public health and is endemic in many tropical and subtropical regions of the world. It affects around 12 million people of the world and 350 million are estimated to be prone to the diseases, of which around 1.7 million people will be infected each year.[1–3] Infection by various strains of Leishmania causes a wide spectrum of disease in humans, with many different clinical manifestations, i.e., cutaneous, mucocutaneous and visceral. The visceral form of Leishmaniasis, commonly known as kala-azar, is caused by the parasite *Leishmania donovani*, which affects 61 out of the 88 countries worldwide. It attacks the phagocytic cells of the spleen, liver and bone marrow, and is fatal in more than 90% of the untreated cases. The parasitic protozoan is digenetic and has two distinct stages in its life cycle. The motile flagellated promastigote stage lives in the alimentary canal of the sandfly vector, which, by inoculation, transmits the promastigotes into the mammalian host, where they enter macrophages differentiating and multiplying into non-motile amastigotes.[4]

There is still no effective vaccine for Kala-azar and chemotherapy remains the most effective control measure. The drugs for the treatment of leishmaniasis are sodium stibogluconate (Pentostam) and meglumine antimonate (Glucantime), despite the fact that they exhibit renal and cardiac toxicity. Although new drugs, i.e., amphotericin B and its lipid complex, are quite effective, they are expensive and out of reach of poor people.[2,5] Newly introduced first orally active drug miltefosine, a phosphocholine analogue, is quite effective in presenting severe gastrointestinal problems and also shows teratogenic effects and cannot be used in pregnant women.[6,7] The search for new drugs continues, with bisphosphonates, for example, risedronate and pamidronate. It is also known that these drugs also contribute to increased co-infections leishmaniasis-AIDS. No treatment has proven to be effective in achieving radical cure of visceral leishmaniasis when it is associated with HIV infection. Therefore, development of more effective and safer chemotherapeutic agents for treating Leishmaniasis seems to be desirable.[8] The pharmaceutical industry has shown little interest in drug discovery for parasitic disease due to a lack of financial incentive. There is also lack of interest among researchers in the developed nations as it is a disease of the developing countries.

Compounds of both synthetic and natural origin comprising a diverse group of chemical structures have been reported as antileishmanial agents. These include mostly the nitrogen heterocycles as quinolines,[9] acridines,[10] phenothiazines,[11] pyrimidines,[12] purines,[13]

Keywords: Lieshmania; Dihydrofolate reductase; Dihydropyrido[2,3-*d*]pyrimidine; Uracil.
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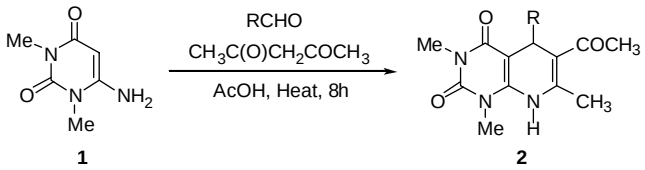
bis-benzamidines,[14] pyrazolo[3,4-*b*]pyridine,[15] benzothiazoles,[16] and imidazolidine.[17] Other classes of compounds include buparvaquone-oxime,[18] bisbenzamidines,[19] chalcones,[20] quinines,[21] amino acid esters and amides,[22] amino alcohols,[23] alkyl phospholipids,[24] ether phospholipids,[25] sulfanilamides,[26] artemisinin[27] and certain platinum complexes.[28]

Uracil derivatives are versatile building blocks for the synthesis of nitrogen-containing heteroaromatic species of biological importance.[29] Pyrazolopyridines,[30] pyrimidopyrimidines,[31] pyridopurines,[32] pyrazolo-pyrimidines[33] and xanthine derivatives[34] have all been prepared by the functionalization of these important heterocyclic building blocks, the structures of which are interesting in their own right, as well as being biologically active pyrimidine nucleosides. The diverse range of biological activities of uracil derivatives in parasitic chemotherapy has stimulated considerable interest in their synthesis.

Dihydrofolate reductase (DHFR) is an important target site in most of the parasitic diseases. Most of the clinically used DHFR inhibitors show less selectivity for leishmanial enzymes.[35] This is due to the fact that the gene for pteridine reductase (PTR1) is amplified in some leishmanial mutants. PTR1 can reduce pterins and folates, and therefore act as a bypass for DHFR inhibition. This implies that antifolate drugs must simultaneously target both DHFR and PTR1 to be successful antileishmanials.[36]

A number of compounds having pteridine, quinazoline and pyrimidine moieties are reported to be potent inhibitors of PTR1 in *Leishmania*.[37] Based on these observations we hypothesized to synthesize pyrido[2,3-*d*]pyrimidines in which one N atom of pteridine was replaced by carbon and in comparison to quinazoline it has one carbon atom replaced by a nitrogen atom which could also act as a PTR1 inhibitor. Earlier reports have shown that pyrido[2,3-*d*]pyrimidine is a potent inhibitor of dihydrofolate reductase.[38] Due to the same reason, the synthesized compounds can act as inhibitors of PTR1, as well as inhibitors of DHFR, and thus could be potential antileishmanial agents.



Scheme 1.

As part of our ongoing program devoted to the synthesis of diverse heterocycles of biological interest,[39] we concentrated our investigations on dihydropyrido[2,3-*d*]pyrimidines[40] due to their broad range of biological activities. In this communication, we have reported the antileishmanial activity of these synthesized compounds.

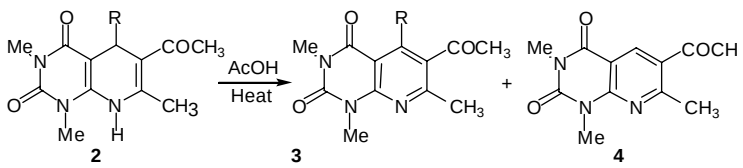
2. Chemistry

Dihydropyrido[2,3-*d*]pyrimidines (**2a–r**) are synthesized in high yields by heating a mixture of 6-amino-1,3-dimethyl uracil, different aldehydes and acetyl acetone in acetic acid (Scheme 1), at 110 °C for 8 h.

Initially after 8 h high yields of dihydropyrido[2,3-*d*]-pyrimidines (**2**) are obtained and oxidized products (**3** and **4**) are either not formed or are formed in a very negligible amount in some cases. As the reaction time is increased, oxidation of the dihydropyrido[2,3-*d*]pyrimidine (**2**) takes place with the formation of dearylated product (**4**) and normal dehydrogenated product (**3**), The amount of 3 and 4 goes on increasing with time (Scheme 2). All the synthesized compounds are well-characterized by spectroscopic method, such as IR, mass, NMR, and elemental analysis.

2.1. Results and discussion

The in vitro biological activity of dihydropyrido[2,3-*d*]pyrimidines has shown encouraging results (Table 1) against *L. donovani*. Compounds showed a good correlation of activity with the structure and log*P* values. Four compounds (**2d**, **2g**, **2h** and **2i**) showed 100% inhibition against promastigotes, whereas two compounds (**2d**, **2g**) exhibited 100% inhibition in amastigotes at a concentration of 50 μg/mL. When the R group was phenyl, the compound **2a** showed 89% and 72% inhibition of promastigotes, and 71% and 56% inhibition of amastigotes at a concentration of 50 and 10 μg/mL, respectively. Substitution of the phenyl ring with a methyl group at the *para* position, compound **2b** showed an increase in activity exhibiting 97% and 74% inhibition for promastigotes, and 80% and 62% for amastigotes. Substitution of another methyl group at *meta* position, compound **2c** showed a further increase in activity. On substitution of the phenyl ring with an isopropyl group at the *para* position, compound **2d** exhibited 100% activity in both promastigotes and amastigotes at a concentration of 50 μg/mL, and 84% and 94% inhibition at a concentration of 10 μg/mL. Activity of compounds gradually increased on substituting the phenyl ring with methyl, dimethyl and isopropyl groups as with the increase in log*P* value. Substituting



Scheme 2.

Table 1. Antileishmanial in vitro activity against luciferase-promastigote and luciferase-amastigote systems

Compounds	R	log*P*	% inhibition			
			Promastigotes concentration (μg/mL)		Amastigotes concentration (μg/mL)	
			50	10	50	10
2a	C_6H_5	0.25	89.2	72.4	71.4	55.6
2b	4-Me-C_6H_4	0.74	96.8	74.2	80.1	61.9
2c	3,4-diMe-C_6H_3	1.23	98.2	78.4	89.9	72.8
2d	4-$CH(CH_3)_2$-C_6H_4	1.49	100	84.2	100	94.2
2e	4-F-C_6H_4	0.41	96.7	86.3	89.4	66.2
2f	4-Cl-C_6H_4	0.81	99.3	89.2	93.2	68.9
2g	3,4-diCl-C_6H_3	1.37	100	92.4	100	80.3
2h	4-CN-C_6H_4	0.29	100	99.1	97.4	76.1
2i	3-NO_2-C_6H_4	1.91	100	93.8	81.6	68.5
2j	4-NO_2-C_6H_4	1.91	100	92.6	88.9	75.7
2k	3-OCH_3-C_6H_4	0.13	84.2	64.1	81.2	46.6
2l	4-OCH_3-C_6H_4	0.13	83.2	68.1	79.6	52.4
2m	3,4-diOCH_3-C_6H_3	0.0	69.2	56.8	54.6	42.5
2n	3,4,5-TriOCH_3-C_6H_2	−0.13	52.6	42.4	38.6	NI
2o	4-OH-C_6H_4	−0.14	59.8	48.4	53.6	42.1
2p	4-COOH-C_6H_4	−0.19	54.6	45.2	NI	NI
2q	2-Furan	−1.20	56.9	32.4	35	22
2r	CH_3	−0.94	59.4	45.7	42.4	26.4
3f	4-Cl-C_6H_4	2.53	55.6	38.4	32.4	ND
3l	4-OCH_3-C_6H_4	1.84	43.3	28.6	12.4	ND
3h	4-CN-C_6H_4	2.00	45.6	ND	28.5	ND
4	—	0.29	38.6	ND	22.1	ND
	SSG®		a	—	21	ND
	Pentamidine®		b	—	90–99	30

a: SSG (sodium stilbogluconate) shows 40–50% inhibition at 500 μg/mL.
b: Pentamidine shows 85–90% inhibition at 0.5 μg/mL.
ND, not determined; NI, no inhibition.

the phenyl ring with groups as fluoro, the compound **2e** showed ~97% and ~86% inhibition in promastigotes at a concentration of 50 and 10 μg/mL, respectively. Compound **2e** showed ~89% and ~66% inhibition in amastigotes at a concentration of 50 and 10 μg/mL, respectively. Replacement of the fluoro group with chloro group the compound **2f** exhibited a further increase in activity in both promastigotes and amastigotes. On disubstituting the phenyl ring with chloro group at 3 and 4 positions, the compound **2g** showed 100% inhibition in both promastigotes and amastigotes at a concentration of 50 μg/mL and inhibited 92% and 80% growth at a concentration of 10 μg/mL. In case of compounds having groups as fluoro and chloro, the activity increased on going from fluoro to chloro and increased further on disubstitution with the increase in log*P* value. On substituting the phenyl ring with a cyano group at the *para* position the compound **2h** showed 100% and 99% inhibition against promastigotes, and 88% and 76% inhibition against amastigotes at a concentration of 50 and 10 μg/mL. Substituting the phenyl ring with the nitro group at *meta* or *para* positions, the compounds **2i** and **2j** have shown 100% inhibition in promastigotes, and 82% and 89% inhibition against amastigotes at a concentration of 50 μg/mL. At a concentration of 10 μg/mL, the compounds **2i** and **2j** showed 92% and 93% inhibition against promastigotes, and 68% and 76% inhibition against amastigotes. Substituting the phenyl ring with methoxy group at the *meta* (**2k**) or *para* position (**2l**) reduced the activity against both promastigotes and amastigotes. Di or tri substitution of the methoxy group in the phenyl ring (**2m** and **2n**) reduced the activity further and the activity decreased with an increase in substitution. Substitution of the methoxy group on the phenyl ring reduced the log*P* value of the compounds, thus reducing the activity. Di or tri substitution of the methoxy group decreased further the log*P* value, showing a decrease in activity. Substitution of the phenyl ring with hydroxyl (**2o**) or carboxyl group (**2p**) reduced the log*P* value of compounds, reducing the activity. Replacement of the phenyl ring with heterocyclic ring as furan (**2q**) or with methyl group (**2r**), the log*P* value of compounds decreased to a greater amount, showing a lower activity. When the dihydropyridine rings of the compounds **2f**, **2l** and **2h** were oxidized to the corresponding pyridine rings the activity of compounds **3f**, **3l** and **3h** was reduced to a great extent. The results emphasize that, in general, the activity increased when chloro, cyano, nitro and methyl are in the aromatic ring. Compounds exhibited a lower activity when methoxy, hydroxy, or carboxy groups are present in the aromatic ring. The activity increased or decreased on di or tri substituting the groups, which increase or decrease the activity. The dihydro pyridine moiety plays an important role in activity as when the dihydro ring was oxidized to the corresponding pyridine ring the activity was reduced to a great extent.

3. Conclusion

Leishmania is a disease of developing countries. The majority of antileishmanial chemotherapy relies on antimonials and benzamidines, which are highly toxic. Therefore, development of more effective and safer chemotherapeutic agents for treating Leishmaniasis remains desirable. There is also lack of interest among those in the pharmaceutical industry in the discovery of a new antileishmanial agent due to a lack of financial incentives. The synthesized dihydropyrido[2,3-*d*]pyrimidines have shown good in vitro activity. These compounds are new leads in antileishmanial chemotherapy and are very useful for further optimization work on the same.

4. Experimental section

IR spectra were recorded on Beckman Aculab-10, Perkin Elmer 881 and FTIR 8210 PC, Shimadzu spectrophotometers either on KBr discs or in neat. Nuclear magnetic resonance (NMR) spectra were recorded on either Bruker Avance DRX-300 MHz or Bruker DPX 200 FT spectrometers using TMS as an internal reference. FAB mass spectra were recorded on JEOL SX 102/DA 6000 mass spectrometer using argon/xenon (6 kV, 10 mA) as the FAB gas. Chemical analysis was carried out on a Carlo-Erba-1108 instrument. The melting points were recorded on an electrically heated melting point apparatus and are uncorrected.

4.1. Antileishmanial activity

Luciferase transfected *L. donovani* promastigotes (MHOM/IN/80/Dd-8, obtained from Imperial College, London), which are more stable under the influence of G 418[18], were maintained at 25 ± 1 °C in Medium 199 (SIGMA Chemical, USA) supplemented with 10% Fetal Calf Serum (Gibco). The in vitro effect of compounds on the growth of promastigotes was assessed by monitoring the luciferase activity of viable cells after treatment. The transgenic promastigotes of late log phase were seeded at 5×10^5/100 μL medium 199/well in 96-well flat-bottomed microtitre (MT) plates (CELLSTAR) and incubated for 72 h in medium alone or in the presence of serial dilutions of drugs (250 ng/mL to 10 μg/mL) in DMSO.[18] Parallel dilutions of DMSO were used as controls. After incubation, an aliquot (50 μL) of promastigote suspension was aspirated from each well of a 96-well plate and mixed with an equal volume of Steady Glo$^{(R)}$ reagent (Promega) and luminescence was measured in luminometer. The values were expressed as RLU (relative luminescence unit).

For assessing the activity of compounds against amastigote stage of the parasite, mouse macrophage cell line (J-774A.1) infected with promastigotes expressing luciferase firefly reporter gene was used. Cells were seeded in a 96-well plate (5×10^4 cells/100 μL/well) in RPMI-1640 containing 10% foetal calf serum and the plates were incubated at 37 °C in a CO_2 incubator. After 24 h, the medium was replaced with fresh medium containing stationary-phase promastigotes (2.5×10^5/100 μL/well). Promastigotes invade the macrophage and are transformed into amastigotes. The test material in appropriate concentrations (50 and 10 μg/mL) in complete medium was added after replacing the previous medium and the plates were incubated at 37 °C in a CO_2 incubator for 24 h or more. After incubation, the drug containing medium was decanted and 50 μL PBS was added to each well and mixed with an equal volume of the steady Glo reagent. After gentle shaking for 1–2 min, the reading was taken in a luminometer.

4.2. General procedure for the synthesis of 6-acetyl-5-(aryl/alkyl)-1,3,7-trimethyl-5,8-dihydro-1*H*-pyrido[2,3-*d*]pyrimidine-2,4-dione (2a–2r)

6-Amino-1,3-dimethyl uracil (**I**) (0.3 mmol), acetyl acetone (0.35 mmol), and aromatic or aliphatic aldehyde (0.35 mmol) were heated in acetic acid (15 ml) for 8 h to obtain dihydropyrido[2,3-*d*]pyrimidines (**2a–r**). The mixture was allowed to cool and water (50 mL) was added. The precipitated solid was washed with water and dried. Recrystallization from ethanol gave light yellow or white crystals of the desired compound.

4.2.1. 6-Acetyl-5-phenyl-1,3,7-trimethyl-5,8-dihydro-1*H*-pyrido[2,3-*d*]pyrimidine-2,4-dione (2a). MS: 326 (M+1); IR (KBr) 3005, 2953, 1708, 1676, 1619, 1514, 1388 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm) 7.26 (s, 1H), 7.24 (d, 2H, J = 6.8 Hz), 7.16 (d, 2H, J = 6.8 Hz), 6.05 (s, 1H, NH), 5.02 (s, 1H), 3.44 (s, 3H, NMe), 3.28 (s, 3H, NMe), 2.45 (s, 3H), 2.15 (s, 3H); ^{13}C ($CDCl_3$, 50 MHz) 199.6, 161.4, 150.7, 142.1, 141.8, 137.2, 135.4, 127.8, 127.1, 124.6, 91.6, 37.5, 29.9, 28.7, 28.1, 19.8; Anal. Calcd for $C_{18}H_{19}N_3O_3$: C, 66.45; H, 5.89; N, 12.91. Found: C, 66.25; H, 6.12; N, 12.84.

4.2.2. 6-Acetyl-1,3,7-trimethyl-5-*p*-tolyl-5,8-dihydro-1*H*-pyrido[2,3-*d*]pyrimidine-2,4-dione (2b). MS: 340 (M+1); IR (KBr) 3006, 2955, 1704, 1661, 1556, 1559, 1514, 1482 cm^{-1};^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm) 7.27 (d, 2H, J = 7.9 Hz), 7.09 (d, 2H, J = 7.9 Hz), 6.01 (s, 1H, NH), 5.09 (s, 1H), 3.44 (s, 3H, NMe), 3.28 (s, 3H, NMe), 2.44 (s, 3H), 2.28 (s, 3H), 2.14 (s, 3H); ^{13}C ($CDCl_3$, 50 MHz) 199.4, 161.4, 150.6, 141.9, 141.7, 137.1, 130.6, 128.1, 126.8, 91.5, 37.4, 34.5, 29.8, 28.7, 28.2, 20.7, 19.8; Anal. Calcd for $C_{19}H_{21}N_3O_3$: C, 67.24; H, 6.24; N, 12.38. Found: C, 67.52; H, 6.34; N, 12.04.

4.2.3. 6-Acetyl-5-(3,4-dimethyl-phenyl)-1,3,7-trimethyl-5,8-dihydro-1*H*-pyrido[2,3-*d*]pyrimidine-2,4-dione (2c). MS: 354 (M+1); IR (KBr) 3015, 2945, 1708, 1665, 1548, 1556, 1519, 1472 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm) 7.09 (d, 1H, J = 7.7 Hz), 7.04 (s, 1H), 6.99 (d, 1H J = 7.7 Hz), 6.01 (s, 1H, NH), 5.09 (s, 1H), 3.44 (s, 3H, NMe), 3.28 (s, 3H, NMe), 2.45 (s, 3H), 2.14 (s, 3H), 2.08 (s, 6H, $2CH_3$); ^{13}C ($CDCl_3$, 50 MHz) 199.3, 161.4, 134.9, 150.6, 141.8, 141.5, 136.9, 136.2, 133.8, 130.2, 129.3, 125.6, 91.6, 38.3, 29.7, 28.6, 28.2, 20.6, 20.3, 19.8; Anal. Calcd for $C_{20}H_{23}N_3O_3$: C, 67.97; H, 6.56; N, 11.89. Found: C, 67.72; H, 6.34; N, 12.04.

4.2.4. 6-Acetyl-5-(4-isopropyl-phenyl)-1,3,7-trimethyl-5,8-dihydro-1*H*-pyrido[2,3-*d*]pyrimidine-2,4-dione (2d). MS: 368 (M+1); IR (KBr) 3020, 2930, 1709, 1675, 1554, 1541,

1517, 1460 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm) 7.38 (d, 2H, J = 7.6 Hz), 7.16 (d, 2H, J = 7.6 Hz), 5.98 (s, 1H, NH), 5.11 (s, 1H), 3.44 (s, 3H, NMe), 3.28 (s, 3H, NMe), 3.18–3.23 (m, 1H), 2.43 (s, 3H, $COCH_3$), 2.26 (s, 3H, CH_3), 1.24 (s, 6H, $2CH_3$); ^{13}C ($CDCl_3$, 50 MHz) 199.5, 161.5, 150.8, 147.5, 142.1, 141.4, 137.1, 127.5, 126.5, 113.9, 91.4, 38.0, 33.7, 29.8, 28.8, 28.2, 23.9, 19.8; Anal. Calcd for $C_{21}H_{25}N_3O_3$: C, 68.64; H, 6.86; N, 11.44. Found: C, 68.52; H, 6.54; N, 11.04.

4.2.5. 6-Acetyl-5-(4-fluoro-phenyl)-1,3,7-trimethyl-5,8-dihydro-1*H*-pyrido[2,3-*d*]pyrimidine-2,4-dione (2e). MS: 344 (M+1); IR (KBr) 3030, 2912, 1705, 1664, 1628, 1514, 1358 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm) 7.37 (d, 2H, J = 8.4 Hz), 6.99 (d, 2H, J = 8.4 Hz), 5.91 (s, 1H, NH), 5.12 (s, 1H), 3.48(s, 3H, NMe), 3.29 (s, 3H, NMe), 2.46 (s, 3H), 2.14 (s, 3H); ^{13}C ($CDCl_3$, 50 MHz) 199.3, 161.4, 158.5, 150.7, 141.8, 141.6, 137.2, 133.1, 128.9, 115.6, 91.5, 37.6, 29.8, 28.7, 28.1, 19.8; Anal. Calcd for $C_{18}H_{18}FN_3O_3$: C, 62.97; H, 5.28; N, 12.24. Found: C, 62.72; H, 5. 44; N, 12.46.

4.2.6. 6-Acetyl-5-(4-chloro-phenyl)-1,3,7-trimethyl-5,8-dihydro-1*H*-pyrido[2,3-*d*]pyrimidine-2,4-dione (2f). MS: 360 (M+1); IR (KBr) 3015, 2946, 1708, 1678, 1623, 1508, 1376 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm) 7.33 (d, 2H, J = 8.3 Hz), 7.25 (d, 2H, J = 8.3 Hz), 6.12 (s, 1H, NH), 5.09 (s, 1H), 3.45 (s, 3H, NMe), 3.28 (s, 3H, NMe), 2.43 (s, 3H), 2.14 (s, 3H); ^{13}C ($CDCl_3$, 50 MHz) 199.5, 161.5, 150.8, 141.8, 141.6, 137.2, 134.4, 130.5, 129.2, 127.6, 91.6, 37.5, 29.8, 28.7, 28.1, 19.8; Anal. Calcd for $C_{18}H_{18}ClN_3O_3$: C, 60.09; H, 5.04; N, 11.68. Found: C, 60.32; H, 5.24; N, 11.84.

4.2.7. 6-Acetyl-5-(3,4-dichloro-phenyl)-1,3,7-trimethyl-5,8-dihydro-1*H*-pyrido[2,3-*d*]pyrimidine-2,4-dione (2g). MS: 394 (M+1); IR (KBr) 3015, 2946, 1708, 1678, 1623, 1508, 1376 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm) 7.38 (d, 1H, J = 7.8 Hz), 7.30 (d, 1H, J = 7.8 Hz), 7.26 (s, 1H), 5.95 (s, 1H, NH), 5.12 (s, 1H), 3.44 (s, 3H, NMe), 3.28 (s, 3H, NMe), 2.44 (s, 3H), 2.14 (s, 3H); ^{13}C ($CDCl_3$, 50 MHz) 199.5, 161.6, 150.7, 141.9, 141.8, 137.2, 135.4, 132.8, 130.8, 127.2, 129.8, 128.7, 91.6, 37.4, 29.7, 28.6, 28.2, 19.8; Anal. Calcd for $C_{18}H_{17}Cl_2N_3O_3$: C, 54.84; H, 4.35; N, 10.66. Found: C, 54.62; H, 4.44; N, 10.34.

4.2.8. 6-Acetyl-1,3,7-trimethyl-2,4-dioxo-1,2,3,4,5,8-hexahydro-pyrido[2,3-*d*]pyrimidine-5-yl-benzonitrile (2h). MS: 351 (M+1); IR (KBr) 3026, 2958, 2155, 1706, 1686, 1619, 1508, 1384 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm) 7.34 (d, 2H, J = 8.4 Hz), 7.12 (d, 2H, J = 8.4 Hz), 6.10 (s, 1H, NH), 5.10 (s, 1H), 3.45 (s, 3H, NMe), 3.28 (s, 3H, NMe), 2.45 (s, 3H), 2.16 (s, 3H); ^{13}C ($CDCl_3$, 50 MHz) 199.5, 161.5, 150.8, 142.1, 141.8, 140.6, 137.2, 129.8, 127.9, 115.5, 118.2, 91.5, 37.5, 29.8, 28.7, 28.2, 19.8; Anal. Calcd for $C_{19}H_{18}N_4O_3$: C, 65.13; H, 5.18; N, 15.99. Found: C, 65.42; H, 5.34; N, 16.14.

4.2.9. 6-Acetyl-1,3,7-trimethyl-5-(3-nitro-phenyl)-5,8-dihydro-1*H*-pyrido[2,3-*d*]pyrimidine-2,4-dione (2i). MS: 371 (M+1); IR (KBr) 3026, 2958, 2155, 1706, 1686, 1619, 1550, 1508, 1384, 1350 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm) 8.09 (d, 1H, J = 8.3Hz), 8.03 (s, 1H), 7.87 (d, 1H J = 8.3 Hz), 7.49 (t, 1H, J = 4.8 Hz), 6.25 (s, 1H, NH), 5.26 (s, 1H), 3.51 (s, 3H, NMe), 3.28 (s, 3H, NMe), 2.49 (s, 3H), 2.16 (s, 3H); ^{13}C ($CDCl_3$, 50 MHz) 199.4, 161.5, 150.8, 146.2, 142.0, 141.8, 137.3, 134.8, 128.6, 124.2, 140.2, 119.6, 91.7, 37.8, 29.9, 28.7, 28.2, 19.8; Anal. Calcd for $C_{18}H_{18}N_4O_5$: C, 58.37; H, 4.90; N, 15.13. Found: C, 58.52; H, 4.64; N, 15.04.

4.2.10. 6-Acetyl-1,3,7-trimethyl-5-(4-nitro-phenyl-5,8-dihydro-1*H*-pyrido[2,3-*d*]pyrimidine-2,4-dione (2j). MS: 371 (M+1); IR (KBr) 3028, 2960, 2150, 1709, 1685, 1610, 1508, 1382, 1354 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm) 8.07 (d, 2H, J = 8.4Hz), 7.77 (d, 2H, J = 8.4 Hz), 6.15 (s, 1H, NH), 5.18 (s, 1H), 3.48 (s, 3H, NMe), 3.28 (s, 3H, NMe), 2.49 (s, 3H), 2.14 (s, 3H); ^{13}C ($CDCl_3$, 50 MHz) 199.4, 161.5, 150.7, 143.2, 142.1, 141.8, 137.6, 137.2, 119.8, 121.5, 91.4, 37.4, 29.8, 28.7, 28.3, 19.9; Anal. Calcd for $C_{18}H_{18}N_4O_5$: C, 58.37; H, 4.90; N, 15.13. Found: C, 58.62; H, 4.84; N, 15.04.

4.2.11. 6-Acetyl-5-(3-methoxy-phenyl)-1,3,7-trimethyl-5,8-dihydro-1*H*-pyrido[2,3-*d*]pyrimidine-2,4-dione (2k). MS: 356 (M+1); IR (KBr) 3014, 2928, 1710, 1667, 1615, 1515, 1379 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm) 7.16 (d, 1H, J = 8.4 Hz), 7.00 (s, 1H), 6.93 (d, 1H, J = 8.5 Hz), 6.72 (t, 1H, J = 5.2 Hz), 6.10 (s, 1H, NH), 5.12 (s, 1H), 3.81 (s, 3H, OMe), 3.45 (s, 3H, NMe), 3.29 (s, 3H, NMe), 2.45 (s, 3H), 2.15 (s, 3H). ^{13}C ($CDCl_3$, 50 MHz): 199.5, 161.5, 158.5, 150.8, 142.0, 141.8, 137.1, 128.6, 128.4, 122.2, 114.2, 111.9, 91.5, 55.1, 37.4, 29.8, 28.7, 28.2, 19.8; Anal. Calcd for $C_{19}H_{21}N_3O_4$: C, 64.21; H, 5.96; N, 11.82. Found: C, 63.98; H, 5.74; N, 11.99.

4.2.12. 6-Acetyl-5-(4-methoxy-phenyl)-1,3,7-trimethyl-5,8-dihydro-1*H*-pyrido[2,3-*d*]pyrimidine-2,4-dione (2l). MS: 356 (M+1); IR (KBr) 3025, 2953, 1705, 1682, 1619, 1502, 1388 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm) 7.30 (d, 2H, J = 8.6 Hz), 6.81 (d, 2H, J = 8.6 Hz), 6.10 (s, 1H, NH), 5.07 (s, 1H), 3.74 (s, 3H, OMe), 3.44 (s, 3H, NMe), 3.28 (s, 3H, NMe), 2.43 (s, 3H), 2.14 (s, 3H). ^{13}C ($CDCl_3$, 50 MHz): 199.5, 161.5, 158.5, 150.8, 142.0, 141.8, 137.1, 128.8, 128.4, 113.9, 91.5, 55.1, 37.4, 29.8, 28.7, 28.2, 19.8; Anal. Calcd for $C_{19}H_{21}N_3O_4$: C, 64.21; H, 5.96; N, 11.82. Found: C, 64.05; H, 6.24; N, 11.94.

4.2.13. 6-Acetyl-5-(3,4-dimethoxy-phenyl)-1,3,7-trimethyl-5,8-dihydro-1*H*-pyrido[2,3-*d*]pyrimidine-2,4-dione (2m). MS: 386 (M+1); IR (KBr) 3025, 2953, 1705, 1682, 1619, 1502, 1388 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm) 7.02 (d, 1H, J = 7.8 Hz), 6.92 (d, 1H, J = 7.8 Hz), 6.84 (s, 1H), 6.10 (s, 1H, NH), 5.06 (s, 1H), 3.74 (s, 6H, 2OMe), 3.44 (s, 3H, NMe), 3.28 (s, 3H, NMe), 2.43 (s, 3H), 2.15 (s, 3H); ^{13}C ($CDCl_3$, 50 MHz): 199.5, 161.4, 150.8, 146.3, 142.5, 141.8, 141.6, 137.2, 129.4, 121.8, 115.4, 114.7, 91.7, 54.9, 37.6, 29.8, 28.8, 28.2, 19.9; Anal. Calcd for $C_{20}H_{23}N_3O_5$: C, 62.33; H, 6.01; N, 10.90. Found: C, 62.45; H, 6.23; N, 10.84.

4.2.14. 6-Acetyl-1,3,7-trimethyl-5-(3,4,5-trimethoxy-phenyl)-5,8-dihydro-1*H*-pyrido[2,3-*d*]pyrimidine-2,4-dione (2n). MS: 416 (M+1); IR (KBr) 3025, 2953, 1705, 1682, 1619, 1502, 1388 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm)

6.62 (s, 2H), 5.99 (s, 1H, NH), 5.10 (s, 1H), 3.82 (s, 6H, 2OMe), 3.79 (s, 3H, OMe), 3.45 (s, 3H, NMe), 3.31 (s, 3H, NMe), 2.46 (s, 3H), 2.17 (s, 3H); ^{13}C ($CDCl_3$, 50 MHz) 199.5, 161.5, 149.2, 150.8, 142.0, 141.8, 137.1, 130.2, 128.4, 105.4, 91.5, 61.4, 56.8, 37.4, 29.8, 28.7, 28.2, 19.8. Anal. Calcd for $C_{21}H_{25}N_3O_6$: C, 60.71; H, 6.07; N, 10.11. Found: C, 60.39; H, 5.84; N, 10.34.

4.2.15. 6-Acetyl-5-(4-hydroxy-phenyl)-1,3,7-trimethyl-5,8-dihydro-1*H*-pyrido[2,3-*d*]pyrimidine-2,4-dione (2o). MS: 342 (M+1); IR (KBr) 3420, 2953, 1709, 1682, 1502, 1388 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm) 7.19 (d, 2H, J = 8.5 Hz), 6.74 (d, 2H, J = 8.5 Hz), 5.00 (s, 1H), 3.48 (s, 3H), 3.21 (s, 3H), 2.43 (s, 3H), 2.15 (s, 3H); ^{13}C ($CDCl_3$, 50 MHz) 199.5, 161.5, 158.5, 150.8, 142.0, 141.8, 137.1, 128.6, 128.2, 113.7, 91.5, 37.4, 29.8, 28.7, 28.2, 19.8. Anal. Calcd for $C_{18}H_{19}N_3O_4$: C, 63.33; H, 5.61; N, 12.31. Found: C, 63.61; H, 5.82; N, 12.58.

4.2.16. 4-(6-Acetyl-1,3,7-trimethyl-2,4-dioxo-1,2,3,4,5,8-hexa-hydro-pyrido[2,3-*d*]pyrimidin-5-yl)-benzoic acid (2p). MS: 370 (M+1); IR (KBr) 3446, 2923, 1704, 1678, 1509, 1382 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm) 7.97 (d, 2H, J = 8.9 Hz), 7.46 (d, 2H, J = 8.9 Hz), 5.18 (s, 1H), 3.54 (s, 3H), 3.27 (s, 3H), 2.45 (s, 3H), 2.13 (s, 3H); ^{13}C ($CDCl_3$, 50 MHz) 199.5, 161.5, 150.8, 144.5, 142.0, 141.8, 137.1, 134.5, 131.8, 126.4, 91.5, 37.4, 29.8, 28.7, 28.2, 19.8; Anal. Calcd for $C_{19}H_{19}N_3O_5$: C, 61.78; H, 5.18; N, 11.38. Found: C, 61.56; H, 5.34; N, 11.62.

4.2.17. 6-Acetyl-5-furan-2-yl-1,3,7-trimethyl-5,8-dihydro-1*H*-pyrido[2,3-*d*]pyrimidine-2,4-dione (2q). MS: 316 (M+1); IR (KBr) 2948, 2936, 1704, 1656, 1532, 1512, 1432, 1373 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm) 7.24 (d, 1H, J = 4.8 Hz), 6.34 (t, 1H, J = 3.2Hz), 6.22 (d, 1H, J = 4.5 Hz), 6.07 (s, 1H, NH), 5.25 (s, 1H), 3.49 (s, 3H, NMe), 3.34 (s, 3H, NMe), 2.40 (s, 3H), 2.28 (s, 3H). ^{13}C ($CDCl_3$, 50 MHz): 199.2, 161.4, 152.5, 150.8, 142.0, 141.8, 137.1, 141.2, 110.2, 105.6, 91.5, 55.1, 37.4, 29.8, 28.7, 28.2, 19.8. Anal. Calcd for $C_{16}H_{17}N_3O_4$: C, 60.94; H, 5.43; N, 13.33. Found: C, 60.67; H, 5.24; N, 13.11.

4.2.18. 6-Acetyl-1,3,5,7-tetramethyl-5,8-dihydro-1*H*-pyrido[2,3-*d*]pyrimidine-2,4-dione (2r). MS: 264 (M+1); IR (KBr) 2963, 2924, 1708, 1665, 1544, 1502, 1428, 1368 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm) 5.97 (s, 1H, NH), 4.02 (dd, 1H, J = 6.8 Hz), 3.49 (s, 3H, NMe), 3.34 (s, 3H, NMe), 2.34 (s, 3H), 2.28 (s, 3H), 1.13 (s, 3H); ^{13}C ($CDCl_3$, 50 MHz) 199.7, 161.6, 150.9, 141.9, 141.1, 139.6, 95.5, 30.4, 29.9, 28.8, 28.2, 19.8, 15.2; Anal. Calcd for $C_{13}H_{17}N_3O_3$: C, 59.30; H, 6.51; N, 15.96. Found: C, 59.53; H, 6.34; N, 15.74.

4.3. General procedure for the synthesis of 6-acetyl-5-(aryl)-1,3,7-trimethyl-1*H*-pyrido[2,3-*d*]pyrimidine-2,4-dione (3) and 6-acetyl-1,3,7-trimethyl-1*H*-pyrido[2,3-*d*]pyrimidine-2,4-dione (4)

6-Amino-1,3-dimethyl uracil (**I**) (0.3 mmol), acetyl acetone (0.35 mmol) and aryl aldehyde (0.35 mmol) were heated in acetic acid (15 mL) for 48 h. The mixture was allowed to cool and water (50 mL) was added. The reaction mixture was extracted with chloroform and dried over sodium sulfate to give the crude product, which was purified by column chromatography (hexane/ethyl acetate; 7:3) to give **2**, **3**, and **4**.

4.3.1. 6-Acetyl-5-(4-chloro-phenyl)-1,3,7-trimethyl-1*H*-pyrido[2,3-*d*]pyrimidine-2,4-dione (3f). MS: 358 (M+1); IR (KBr) 3015, 2962, 1712, 1652, 1558, 1541, 1508, 1482 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm) 7.54 (d, 2H, J = 8.2 Hz), 7.43 (d, 2H, J = 8.2 Hz), 3.74 (s, 3H, NMe), 3.32 (s, 3H, NMe), 2.55 (s, 3H, $COCH_3$), 1.82 (s, 3H, CH_3); ^{13}C ($CDCl_3$, 50 MHz) 204.4, 160.3, 159.6, 151.2, 150.4, 149.5, 139.7, 132.4, 130.2, 127.4, 120.9, 105.5, 31.7, 29.9, 28.3, 23.5; Anal. Calcd for $C_{18}H_{16}N_3O_3$: C, 60.42; H, 4.51; N, 11.74. Found: C, 60.52; H, 4.44; N, 12.04.

4.3.2. 4-(6-Acetyl-1,3,7-trimethyl-2,4-dioxo-1,2,3,4-tetra-hydro-pyrido[2,3-*d*]pyrimidin-5-yl)-benzonitrile (3h). MS: 349 (M+1); IR (KBr) 3008, 2948, 2150, 1708, 1675, 1546, 1553, 1514, 1484 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm) 7.18 (d, 2H, J = 7.9 Hz), 7.01 (d, 2H, J = 7.9 Hz), 3.72 (s, 3H, NMe), 3.38 (s, 3H, NMe), 2.52 (s, 3H), 1.86 (s, 3H); ^{13}C ($CDCl_3$, 50 MHz) 204.5, 160.4, 159.6, 151.2, 150.6, 149.6, 146.7, 130.2, 129.3, 120.4, 115.4, 111.5, 105.5, 31.8, 29.9, 28.4, 23.6; Anal. Calcd for $C_{19}H_{16}N_4O_3$: C, 65.51; H, 4.63; N, 16.08. Found: C, 65.72; H, 4.34; N, 16.24.

4.3.3. 6-Acetyl-5-(4-methoxy-phenyl)-1,3,7-trimethyl-1*H*-pyrido[2,3-*d*]pyrimidine-2,4-dione (3l). MS: 354 (M+1); IR (KBr) 3005, 2957, 1706, 1665, 1558, 1558, 1513, 1480 cm^{-1};^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm) 7.14 (d, 2H, J = 8.6Hz), 6.97 (d, 2H, J = 8.6 Hz), 3.85 (s, 3H, OMe), 3.76 (s, 3H, NMe), 3.35 (s, 3H, NMe), 2.53 (s, 3H, $COCH_3$), 1.85 (s, 3H, CH_3); ^{13}C ($CDCl_3$, 50 MHz) 204.4, 160.3, 159.8, 158.4, 151.2, 150.8, 149.9, 136.7, 129.6, 120.7, 105.2, 113.4, 55.3, 31.7, 29.9, 28.3, 23.5; Anal. Calcd for $C_{19}H_{19}N_3O_4$: C, 64.58; H, 5.42; N, 11.89. Found: C, 64.82; H, 5.34; N, 12.04.

4.3.4. 6-Acetyl-1,3,7-trimethyl-1*H*-pyrido[2,3-*d*]-pyrimidine-2,4-dione (4). MS: 248 (M+1); IR (KBr) 2960, 2928, 1702, 1663, 1564, 1509, 1440, 1371 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm) 6.81 (s, 1H), 3.68 (s, 3H), 3.43 (s, 3H), 2.76 (s, 3H), 2.52 (s, 3H); ^{13}C ($CDCl_3$, 50 MHz) 162.8, 162.3, 153.4, 151.8, 122.2, 107.2, 30.2, 28.5, 25.0, 22.6; Anal. Calcd for $C_{12}H_{13}N_3O_3$: C, 58.29; H, 5.30; N, 17.00. Found: C, 58.48; H, 5.12; N, 17.24.

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