Tetrahedron Letters 55 (2014) 1796–1801

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

A convenient synthesis of novel 5-aryl-pyrido[2,3-*d*]pyrimidines and screening of their preliminary antibacterial properties

Lakhinath Saikia [a,b], Babulal Das [c], Pranjal Bharali [d], Ashim Jyoti Thakur [a,*]

[a] Department of Chemical Sciences, Tezpur University (A Central University), Tezpur, Napaam 784028, Assam, India
[b] Department of Chemistry, Rajiv Gandhi University (A Central University), Rono Hills, Doimukh 791112, Arunachal Pradesh, India
[c] Department of Chemistry, Indian Institute of Technology, Guwahati 781039, Assam, India
[d] Department of Molecular Biology and Biotechnology, Tezpur University (A Central University), Tezpur, Napaam 784028, Assam, India

ARTICLE INFO

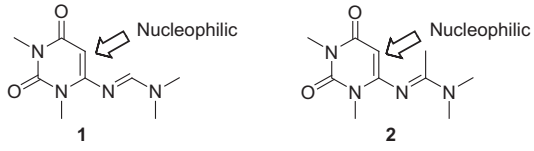
Article history:
Received 26 October 2013
Revised 26 January 2014
Accepted 27 January 2014
Available online 3 February 2014

Keywords:
Uracil
Pyrido[2,3-*d*]pyrimidine
Amidine
Antibacterial
Diels–Alder

ABSTRACT

Exploiting the diene nature of 6-[1-aza-2-(dimethylamino)prop-l-enyl]-1,3-dimethyluracil (**2**), novel 5-aryl-pyrido[2,3-*d*]pyrimidines (**5a**–**h**) have been synthesized. The reaction proceeds through a triene intermediate whose structure has been conclusively established by single crystal X-ray analysis. Role of water is intriguing as the reaction can be stopped at the intermediate stage. Synthesized compounds have been screened for possible antibacterial properties and results showed modest activity.

Nature has been more perspicacious than the organic chemists in designing effective bioactive molecules. Nevertheless, mimicking nature, synthetic organic chemists also put their best efforts to find out new potent molecules. The nucleo base pyrimidine and its derivatives[1] are not only powerful biological and medicinal entities,[2] but their annulated derivatives, pyrido[2,3-*d*]pyrimidines are also associated with interesting pharmacological properties.[3] Recently, in Nature Reviews Drug Discovery,[4] M. H. Flight highlighted the work of Stover et al.[5] where screening of 1.6 million compounds from Pfizer compound library was carried out for antibacterial activity. This screening resulted in the discovery of three pyrido[2,3-*d*]pyrimidine derivatives as potent synthetic antibacterials that targeted the bacterial biotin carboxylase selectively. In a very recent report,[6] A. Zega et al. demonstrated 6-aryl-pyrido[2,3-*d*]pyrimidines as novel ATP-competitive inhibitors of bacterial D-alanine: D-alanine ligase. The need for new and novel antibiotics to combat bacterial drug resistance has led the researchers to give enormous efforts in antibiotic research. Hence, there has been flurry of synthetic and biological activities[3,7] centered on pyrido[2,3-*d*]pyrimidine that attract considerable attentions from chemists and biologists. A plethora of methods[7g–i,8] have been reported for the synthesis of pyrido[2,3-*d*]pyrimidines and related compounds by exploiting the diene character of 6-[(dimethylamino)methylene-amino]-1,3-dimethyluracil (**1**, Fig. 1) via [4+2] cycloaddition reaction with various electron deficient dienophiles. Recently, our group has reported the synthesis of bis-pyrimido[4,5-*d*]pyrimidines from **1** via aza Diels–Alder methodology followed by their preliminary bioactivity.[9] However, the simple acetamidine counterpart of **1**, that is 6-[1-aza-2-(dimethylamino)prop-l-enyl]-1,3-dimethyluracil[10] (**2**, Fig. 1) and its diene behaviour are not well explored so far. In fact, no report of cycloaddition reaction using **2** has been found in the literature and to the best of our knowledge, only a single report[11] of coupling reaction using the 5-iodinated **2** in the presence of palladium catalyst has been found. Therefore, we report here the exploitation of diene behaviour of **2** for the convenient synthesis of 5-aryl-7-methylpyrido[2,3-*d*]pyrimidines and their in vitro antibacterial activity. Formation of 5-aryl-7-methylpyrido[2,3-*d*]pyrimidines is not quite expected as **1** has been found to furnish 5-aryl-5,8-dihydropyrido[2,3-*d*]pyrimidines with nitrostyrenes under similar condition as reported[8f] very recently.

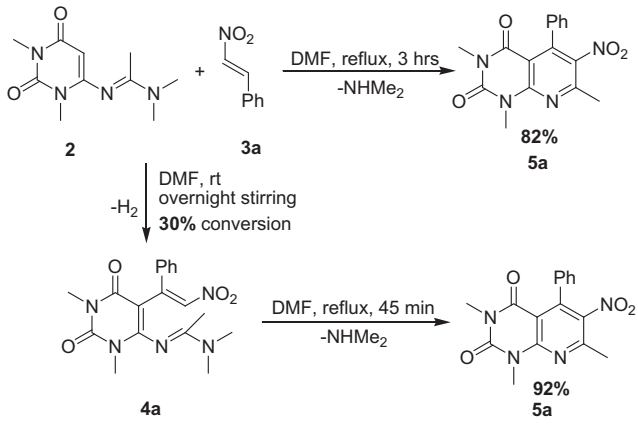


1 2

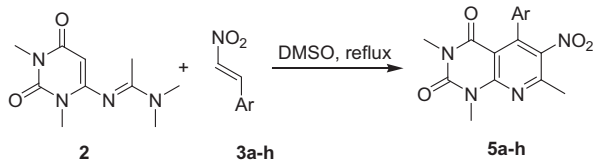
Figure 1. Methylated (**2**) and non methylated amidine (**1**).

* Corresponding author. Tel.: +91 275059; fax: +91 (3712) 267005/6.
E-mail address: ashim@tezu.ernet.in (A.J. Thakur).

http://dx.doi.org/10.1016/j.tetlet.2014.01.128
0040-4039/



Scheme 1. Synthesis of pyrido[2,3-*d*]pyrimidine **5a**.



Scheme 2. Synthesis of pyrido[2,3-*d*]pyrimidines (**5a**–**h**) in one step.

In our initial attempt to investigate the interesting diene behaviour of the methylated amidine (**2**) in [4+2] cycloaddition reaction, we treated **2** with β-nitrostyrene (**3a**) (Scheme 1), an electron deficient dienophile. During the process, we observed lower reactivity of **2** towards **3a** than **1**. The percentage conversion of **2** in chloroform was found to be 35% only (stirring at room temperature for 12 h, (entry 5, Table 1) whereas **1** underwent complete conversion within 5 minutes (stiring at 0 °C) as demonstrated in our earlier report.[7e] This lower reactivity of **2** towards *β*-nitrostyrene, in spite of having one extra electron releasing methyl group is contradictory to the general rule of Diels–Alder reaction which suggests enhanced reactivity of **2** than **1**. This might be due to the reason that the presence of this extra methyl group hinders the extent of delocalization starting from the dimethylamino group and hinders the planarity. However, the reaction occurs smoothly in refluxing DMF with complete conversion of **2** within 3 h furnishing 1,3,7-trimethyl-6-nitro-5-phenylpyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (**5a**) in 82% yield. The structure of **5a** has been established using FTIR spectroscopy, MS (ESI), ^{1}H & ^{13}C NMR spectroscopy and elemental analyses. To gain some insight into the mechanism of the reaction, the reaction was monitored at different time intervals using TLC. As the reaction progressed, we noticed an extra spot in TLC with distinctly lower R_f value than that of **5a**, whose intensity decreased gradually over time. The same spot was observed in TLC as the single spot other than the starting compounds when we carried out the reaction at room temperature for 12 h (Scheme 1), although only 30% conversion of the starting materials was observed here. These observations suggest that this extra spot in TLC might be due to the formation of an intermediate which underwent further reaction under the reaction condition to furnish the final product. The intermediate compound responsible for this new spot was isolated using column chromatography and its structure was established as **4a**, a Michael type product by single crystal X-ray analysis. A similar reaction condition was imposed (i.e. refluxed in DMF) to **4a** and to our delight, it offered the same product **5a** within shorter period of time and thus supported the involvement of **4a** as an intermediate.

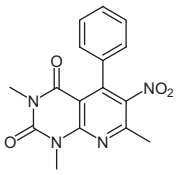
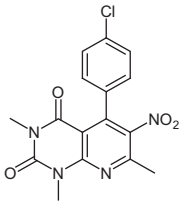
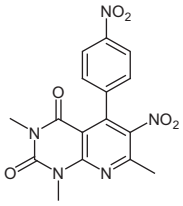
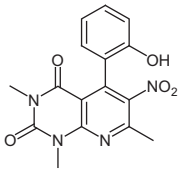
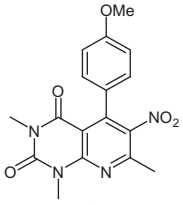
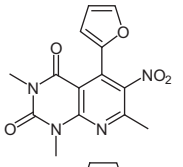
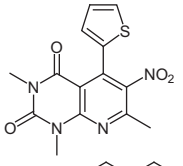
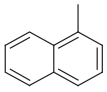
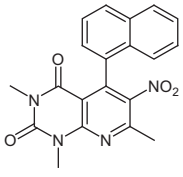
To study the effect of solvents in the formation of **4a** as well as **5a**, the reaction was carried out in different solvents (both polar and non-polar) by stirring the reaction mixture at room temperature for 12 h followed by refluxing it for 3 h. The results are summarized in Table 1. Table 1 reveals that high boiling aprotic polar solvents like DMF and DMSO (Table 1, entries 1 & 2) are suitable for the formation of the final product **5a**, but the rate of conversion towards the intermediate **4a** is quite low. In contrast, water is found to be the best solvent for the formation of the intermediate **4a**, but not so satisfactory for furnishing the final product **5a**. To examine the effect of H_2O, the reaction was then carried out in a 1:1 mixture of **4a**, but not good enough to give **5a** in satisfactory yield. On the basis of all these observations, it can be concluded that H_2O favours the formation of **4a** whereas it disfavours the conversion of **4a** to the product (**5a**). Other solvents like chloroform, acetonitrile, benzene and methanol were not found to be suitable. Among all these solvents checked, DMSO was found to be the best solvent for the synthesis of pyrido[2,3-*d*]pyrimidine (**5a**) in a single step process. Generalization of the reaction was carried out using various *β*-nitrostyrenes (**3a**–**h**) as shown in Scheme 2 and summarized in Table 2. The reaction did not proceed with aliphatic nitroalkenes. The synthesis of **5** was also attempted via a two-step route (Scheme 3) considering the observation that H_2O was the best solvent for the intermediate (**4**) formation step whereas DMF and DMSO were the excellent solvents for the formation of intermediate (**4**) to final product (**5**). It was found that this route was slightly more beneficial than the single step route when the overall isolated yields of **5** were considered. The 2nd step was carried out in refluxing DMF although both DMF & DMSO were found to give almost comparable yield. However, DMF is preferred over DMSO, as DMSO has an extremely unpleasant smell when refluxed. Chloro, nitro, hydroxyl, methoxy and heterocyclic moieties

Table 1
Screening of solvents for Scheme 1

Entry	Solvent	Reaction condition					
		Stirred at rt for overnight (12 h)			Refluxed for 3 h[a]		
		Conversion of **2** (%)	Yield of **4a** (%)	Yield of **5a** (%)	Conversion of **2** (%)	Yield of **4a** (%)	Yield of **5a** (%)
1	DMF	30	100	No product formation	100	<10	82
2	DMSO	32	100	No product formation	100	<10	85
3	H_2O	100	100	No product formation	100	8	22[b]
4	DMF–H_2O (1:1)	100	100	No product formation	100	12	28[b]
5	$CHCl_3$	35	100	No product formation	50	100	NF
7	CH_3CN	38	100	No product formation	68	>90	<10
8	C_6H_6	15	100	No product formation	60	45	18[b]
9	CH_3OH	75	100	No product formation	92	65	<10[b]

[a] Reaction mixture was stirred at room temperature for 12 h followed by reflux for 3 h.
[b] Some other unidentified products were formed.

Table 2
Synthesis of pyrido[2,3-*d*]pyrimidines (**5a–h**)[12] via Scheme 2

Entry	Ar– in (**3**)	Product (**5**)	Time (h)	Yield[a] (%)
1	C_6H_5-**3a**		3	85
2	*p*-ClC_6H_4-**3b**		3	82
3	*p*-$NO_2C_6H_4$-**3c**		3.5	72
4	*o*-HOC_6H_4-**3d**		3.5	75
5	*p*-$MeOC_6H_4$-**3e**		2.5	92
6	 **3f**		2.5	87
7	 **3g**		2.5	90
8	 **3h**		2.5	85

[a] Yields refer to the isolated pure products.

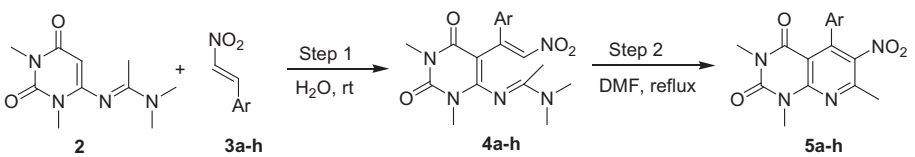
(thiophene and furan) remained undisturbed during the reaction. The results are summarized in Table 3. All the intermediates (**4a–h**) were obtained by simple filtration and used for the 2nd step without any further purification. The structure of all the final products was confirmed by IR spectroscopy, ^1H and ^{13}C NMR spectroscopy (see ESI), elemental analyses and mass spectral data.

Conclusive evidence for the structures of **4a** and **5g** were obtained from single crystal X-ray analyses (Figs. 2 and 3, respectively).

As illustrated in Table 2, in all the reactions with unsymmetrical nitrostyrenes only one regioisomer was obtained. Reactions of **2** with other comparatively less electron deficient dienophiles such as acryl amide, acrylic acid, cinnamaldehyde, cinnamic acid, cinnamamide, benzylideneacetophenone (not shown in Table 2) were also examined under similar reaction conditions, but failed to give product. Although the detailed mechanistic studies were not performed, a plausible mechanism is suggested to rationalize the formation of the product **5** via aza-Diels–Alder pathway followed by elimination of hydrogen molecule, rearrangement and loss of dimethylamine (Scheme 4).

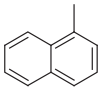
We became enthusiastic by the success of our methodology and this prompted us to extend the methodology to di(nitrostyrene) **6** obtained from terephthaldehyde also (Scheme 5). The reaction was found to be effective, although the yield was low (58%).

The synthesized pyrido[2,3-*d*]pyrimidines (**5a–h** and **7**) were screened for their in vitro antibacterial property against both the Gram positive (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram negative (*Klebsilla pneumonia* and *Escherichia coli*) bacterial strains. The minimum inhibitory concentrations (MIC) of the synthesized compounds were determined (Table 4) by the micro dilution method taking streptomycin sulfate (1 mg/mL) as positive control while sterilized DMSO as negative control. Among the compounds, **5a** exhibited maximum activity against all the tested microorganisms although compound **5h** showed identical activity against *S. aureus* whereas compound **7** possessed identical activity against *K. pneumonia* and *E. coli*. Among all the compounds, **5a** was found to be the best antibacterial agent against a broad spectrum of microorganisms followed by **7** and **5h**. When the activity against gram positive and gram negative bacteria was considered, **5a** retained its best position against gram positive bacteria while **7** shared the top position with it against the gram negative bacteria. The results indicate that introduction of both electron withdrawing (Table 2, entries 2 and 3) and electron releasing (Table 2, entries 4 and 5) groups to the aryl ring at position **5** of the pyrido[2,3-*d*]pyrimidine decreases the activity of the compounds although the effect of electron releasing groups is much higher. Replacement of aryl rings by heteroaryl rings (Table 2, entries 6 and 7) also leads to an increase in MIC values. Compounds with unsubstituted aromatic ring (phenyl, naphthyl in **5a**, **5h** and **7**) at positions 5 offer the best activity. To examine the effect of the $-NO_2$ group at position 6 of pyrido[2,3-*d*]pyrimidines towards the antibacterial activity, the $-NO_2$ group of **5e** (compound with the highest MIC value) was reduced to $-NH_2$ functionality (Scheme 6) and the resulting amine (**8e**) was screened for possible antibacterial activity and to our delight, it showed excellent activity as compared to **5e** (Table 4, entry 11), that is upon conversion of $-NO_2$ to $-NH_2$, the activity



Scheme 3. Synthesis of pyrido[2,3-*d*]pyrimidines (**5a–h**) in two steps.

Table 3
Synthesis of pyrido[2,3-*d*]pyrimidines **5a–h**[13] via Scheme 3 in two steps

Entry	Ar–	Step 1			mp (°C)	Step 2			Overall yield[a] (%)
		Reaction time (h)	Product	Yield[a] (%)		Reaction time	Product	Yield[a] (%)	
1	C_6H_5–	8	4a	100	145	45 min	5a	92	92
2	*p*-ClC_6H_4–	8	4b	100	140	50 min	5b	90	90
3	*p*-$NO_2C_6H_4$–	12	4c	96	172	1.5 h	5c	85	82
4	*o*-HOC_6H_4–	12	4d	98	118	1.5 h	5d	85	83
5	*p*-$MeOC_6H_4$–	7	4e	100	139	40 min	5e	95	95
6		7.5	4f	100	157	45 min	5f	92	92
7		7.5	4g	100	152	45 min	5 g	90	90
8		8.5	4h	100	161	1 h	5 h	88	88

[a] Yields refer to the isolated pure products.

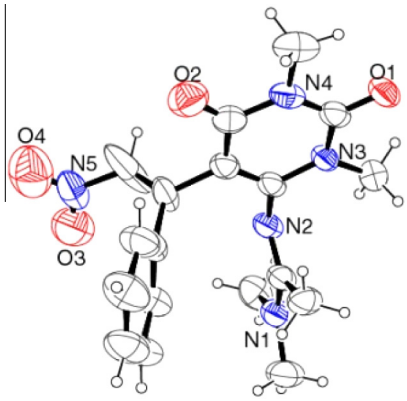


Figure 2. ORTEP diagram of **4a**.

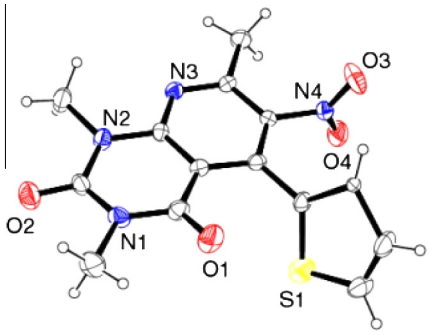
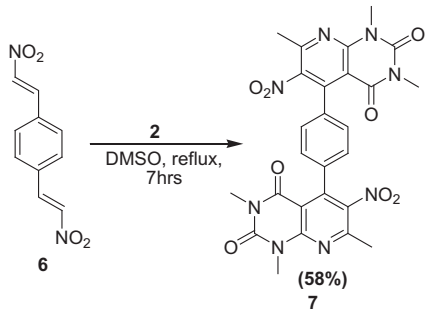


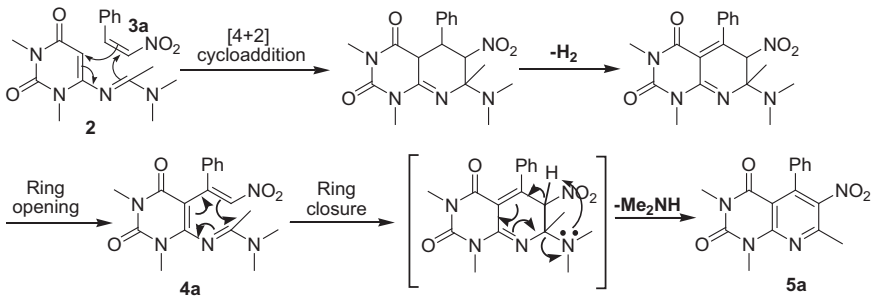
Figure 3. ORTEP diagram of **5g**.



Scheme 5. Synthesis of **7**.

increases enormously. Functionally transformed products (**8a**, **8h** and **9**) of the most active compounds (**5a**, **5h** and **7**) were also screened against bacterial strains and an increase in activity was observed (Table 4, entries 10, 12 and 13). However, the magnitude of increase in activity upon functional transformation here is very less in comparison to that observed in the functional transformation of the least active compound.

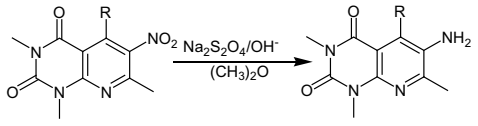
In conclusion, a convenient catalyst-free regioselective synthesis of 5-aryl-pyrido[2,3-*d*]pyrimidines has been accomplished via hetero annulation, where pyridine component carries a methyl substituent at 7-position. The generality of the protocol has been demonstrated by the successful conversion of eight substrates into pyrido[2,3-*d*]pyrimidines **5a–h**. This method allows for the introduction of a high degree of chemical and structural diversity onto the pyrimidine scaffold with tolerability to several groups. Easy access of the starting materials, good yields of the products and

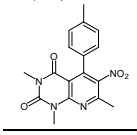


Scheme 4. Plausible mechanism for the formation of **5a**.

Table 4
MIC (in mg mL^{-1}) values of novel 5-Aryl-pyrido[2,3-*d*]pyrimidines (**5a–h**, **7**, **8** and **9**)

Entry	Compounds	Microorganisms			
		Gram positive		Gram negative	
		B. subtilis	*S. aureus*	*K. pneumonia*	*E. coli*
1	**5a**	0.375	0.375	0.75	0.75
2	**5b**	1.8	0.9	1.8	0.9
3	**5c**	2	4	4	4
4	**5d**	3.75	7.5	7.5	7.5
5	**5e**	27	27	27	27
6	**5f**	2.5	1.25	2.5	2.5
7	**5g**	4.75	4.75	4.75	4.75
8	**5h**	1.5	0.375	3	1.5
9	**7**	0.75	0.75	0.75	0.75
10	**8a**	0.375	0.375	0.375	0.375
11	**8e**	1.25	2.5	1.25	1.25
12	**8h**	0.75	0.375	1.75	0.75
13	**9**	0.25	0.25	0.25	0.50



R-	Substrate	Product	Isolated Yield (%)
C_6H_5-	**5a**	**8a**	72
p-$MeOC_6H_4$-	**5e**	**8e**	74
	**5h**	**8h**	68
	**7**	**9**	42

Scheme 6. Reduction of $-NO_2$ to $-NH_2$.[14]

the simplicity of the experimental procedure make it a useful one. Synthesized compounds possess modest activity towards gram positive and gram negative bacteria. Noticeably, functional conversion of the $-NO_2$ group to $-NH_2$ leads to enhanced antibacterial activity.

Acknowledgments

The authors are indebted to the Council of Scientific and Industrial Research (CSIR), New Delhi, India, for financial support to the project CSIR (01(2147)/07/EMR-II).

Supplementary data

Crystallographic information: Crystallographic data (excluding structure factors) for compounds **4a** & **5g** have been deposited with the Cambridge Crystallographic Data Centre as deposition Nos. CCDC 875794 & CCDC 875795 respectively. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (Fax: +44 (0) 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk).

Supplementary data (experimental section, screening for antibacterial activity of the synthesized compounds, ^1H and ^{13}C NMR spectra of compounds) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2014.01.128.

References and notes

1. Saladino, R.; Crestini, C.; Costanzo, G.; Negri, R.; Mauro, E. D. *Bioorg. Med. Chem.* **2001**, *9*, 1249–1253.
2. (a) Roth, H. J.; Kleemann, A. *Pharmaceutical Chemistry. Volume 1: Drug Synthesis*, John Wiley & Sons: New York, 1988.; (b) Jain, K. S.; Chitre, T. S.; Miniyar, P. B.; Kathiravan, M. K.; Bendre, V. S.; Veer, V. S.; Shahane, S. R.; Shishoo, C. J. *Curr. Sci.* **2006**, *90*, 793–803; (c) Burdge, E. L. *Pest Manag. Sci.* **2000**, *56*, 245–248; (d) Tice, C. M. U.S. Patent 5300477, 1994; *Chem. Abstr.* **1994**, *122*, 31543.
3. (a) Hopkins, F. G. *Nature* **1889**, *40*, 335–336; *Nature* **1891**, *45*, 197–198; *Nature* **1892**, *45*, 581–581; (b) Heckler, R. E.; Jourdan, G. P. Eur. Patent 414 386, 1991; *Chem. Abstr.* **1991**, *115*, 71630; (c) Degraw, J. I.; Christie, P. H.; Colwell, W. T.; Sirotnak, F. M. *J. Med. Chem.* **1992**, *35*, 320–324; (d) Matsumoto, J.; Minami, S. *J. Med. Chem.* **1975**, *18*, 74–79; (e) Bulicz, J.; Bertarelli, D. C. G.; Baumert, D.; Fülle, F.; Müller, C. E.; Heber, D. *Bioorg. Med. Chem.* **2006**, *14*, 2837–2849; (f) Huang, H.; Hutta, D. A.; Rinker, J. M.; Hu, H.; Parsons, W. H.; Schubert, C.; DesJarlais, R. L.; Crysler, C. S.; Chaikin, M. A.; Donatelli, R. R.; Chen, Y.; Cheng, D.; Zhou, Z.; Yurkow, E.; Manthey, C. L.; Player, M. R. *J. Med. Chem.* **2009**, *52*, 1081–1099; (g) Wissing, J.; Godl, K.; Brehmer, D.; Blencke, S.; Weber, M.; Habenberger, P.; Stein-Gerlach, M.; Missio, A.; Cotten, M.; Müller, S.; Daub, H. *Mol. Cell. Proteomics* **2004**, *3*, 1181–1193; (h) Kanth, S. R.; Reddy, G. V.; Kishore, K. H.; Rao, P. S.; Narsaiah, B.; Murthy, U. S. N. *Eur. J. Med. Chem.* **2006**, *41*, 1011–1016; (i) Bazgir, A.; Khanaposhtani, M. M.; Soorki, A. A. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5800–5803.
4. Flight, M. H. *Nat. Rev. Drug Disc.* **2009**, *8*. 193–193.
5. Miller, J. R.; Dunham, S.; Mochalkin, I.; Banotai, C.; Bowman, M.; Buist, S.; Dunkle, B.; Hanna, D.; Harwood, H. J.; Huband, M. D.; Karnovsky, A.; Kuhn, M.; Limberakis, C.; Liu, J. Y.; Mehrens, S.; Mueller, W. T.; Narasimhan, L.; Ogden, A.; Ohren, J.; Prasad, J. V. N. V.; Shelly, J. A.; Skerlos, L.; Sulavik, M.; Thomas, V. H.; VanderRoesta, S.; Wang, L.; Wang, Z.; Whitton, A.; Zhu, T.; Stover, C. K. *PNAS* **2009**, *106*(6), 1737–1742.
6. Škedelj, V.; Arsovska, E.; Tomašić, T.; Kroflič, A.; Hodnik, V.; Hrast, M.; Bešter-Rogač, M.; Anderluh, G.; Gobec, S.; Bostock, J.; Chopra, I.; O'Neill, A. J.; Randall, C.; Zega, A. *PLOS One* **2012**, *7*, e39922.
7. (a) Devi, I.; Kumar, B. S. D.; Bhuyan, P. J. *Tetrahedron Lett.* **2003**, *44*, 8307–8310; (b) Youssif, S.; El-Bahaie, S.; Nabih, E. *J. Chem. Research (S)* **1999**, 112–113; (c) Bagley, M. C.; Hughes, D. D.; Lloyd, R.; Powers, V. E. C. *Tetrahedron Lett.* **2001**, *42*, 6585–6588; (d) Nair, V.; Chi, G.; Shu, Q.; Julander, J.; Smee, D. F. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1425–1427; (e) Hagen, H.; Raatz, P.; Walter, H.; Landes, A. Ger. Offen. 1992, DE 4035479 A1 19920514.; (f) Gordeev, M. F.; Patel, D. V.; Wu, J.; Gordon, E. M. *Tetrahedron Lett.* **1996**, *37*, 4643–4646; (g) Thakur, A. J.; Saikia, P.; Prajapati, D.; Sandhu, J. S. *Synlett* **2001**, 1299–1301; (h) Sarma, R.; Borah, K. J.; Dommaraju, Y.; Prajapati, D. *Mol. Divers* **2011**, *15*, 697–705; (i) Walsh, E. B.; Nai-Jue, Z.; Fang, G.; Wamhoff, H. *Tetrahedron Lett.* **1988**, *29*, 4401–4404.
8. (a) Prajapati, D.; Gohain, M.; Thakur, A. J. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3537–3540; (b) Prajapati, D.; Thakur, A. J. *Tetrahedron Lett.* **2005**, *46*, 1433–1436; (c) Saikia, P.; Thakur, A. J.; Prajapati, D.; Sandhu, J. S. *Indian J. Chem.* **2002**, *41B*, 804–807; (d) Gohain, M.; Prajapati, D.; Gogoi, B. J.; Sandhu, J. S. *Synlett* **2004**, 1179–1182; (e) Sarma, R.; Sarmah, M. M.; Prajapati, D. *J. Org. Chem.* **2012**, *77*, 2018–2023; (f) Sarmah, M. M.; Prajapati, D.; Hu, W. *Synlett* **2013**, 471–474.
9. Das, S.; Thakur, A. J.; Medhi, T.; Das, B. *RSC Adv.* **2013**, *3*, 3407–3413.
10. *Procedure for the synthesis of compound 6-[1-aza-2-(dimethylamino)prop-l-enyl]-1,3-dimethyluracil* ***2****:* 6-amino-1,3-dimethyl-pyrimidine-2,4(1*H*,3*H*)-dione (1.55 g, 0.01 mol) and *N*,*N*-Dimethylacetamide dimethyl acetal (DMA-DMA) (1.33 g, 0.01 mol) were refluxed in a round bottomed flask for 1 h and then the reaction mixture was allowed to cool to room temperature. The solid mass thus obtained was recrystallized from ethanol to get **2** as pure product. Yield (2.03 g, 90.62%).
Solid; mp. 152 °C; IR (KBr) (ν_{max}/cm^{-1}) 3025, 2926, 1716, 1673, 1662, 1483; δ_H (400 MHz, $CDCl_3$, TMS) 4.83 (s, 1H), 3.34 (s, 3H), 3.29 (s, 3H), 3.11 (d, *J*=6.8 Hz, 6H), 2.12 (s, 3H) ppm; δ_C (100 MHz, $CDCl_3$, TMS) 163.7, 159.7, 157.7, 152.9, 86.9, 38.6, 37.8, 30.2, 27.7, 15.9 ppm; MS (ESI): *m*/*z* 224 $(M)^+$; Anal. Calcd (%) for $C_{10}H_{16}N_4O_2$: C, 53.56; H, 7.19; N, 24.98. Found: C, 53.57; H, 7.23; N, 24.95%.
11. Roh, Y. H.; Bae, J. W.; Nam, G. S.; Kim, J. H.; Kim, S. H.; Yoon, C. M. *Synth. Commun.* **2000**, *30*, 81–86.
12. *General procedure for the synthesis of* ***5a–h*** *via Scheme 2:* 6-[1-aza-2-(dimethylamino)prop-l-enyl]-1,3-dimethyl-uracil 2 (0.5 mmol) and the nitrostyrene derivative **3a–h** (0.5 mmol) were taken in DMSO (5 mL) and refluxed. After the completion of the reaction as monitored by TLC, DMSO was removed by vacuum distillation. The crude residue was purified to get the pure products **5a–h** by column chromatography using silica gel (100–200 mesh) as adsorbent and Ethyl acetate–Hexane (1:4) as eluent.
1,3,7-Trimethyl-6-nitro-5-phenylpyrido[2,3-d]pyrimidine-2,4(1H,3H)-dione ***(5a)*** *(Table 2, entry 1):* Solid; mp. 180 °C; IR (KBr) (ν_{max}/cm^{-1}) 3455, 2926, 2370, 1716, 1673, 1561, 1483, 1346; δ_H (400 MHz, $CDCl_3$, TMS) 7.16–7.43 (m, 5H), 3.75 (s, 3H), 3.31 (s, 3H), 2.62 (s, 3H) ppm; δ_C (100 MHz, $CDCl_3$, TMS) 153.5, 149.9, 149.8, 145.2, 131.1, 128.1, 127.1, 126.2, 29.3, 27.6, 20.3 ppm; MS (ESI) *m*/*z* 326 $(M)^+$; Anal. Calcd (%) for $C_{16}H_{14}N_4O_4$: C, 58.89; H, 4.32; N, 17.17%. Found: C, 58.91; H, 4.37; N, 17.19%.
13. *General procedure for the synthesis of* ***5a–h*** *via Scheme 3:*
Step 1 (Synthesis of ***4a–h****):* 6-[1-aza-2-(dimethylamino) prop-l-enyl]-1,3-dimethyluracil 2 (0.5 mmol) and the nitrostyrene **3a–h** (0.5 mmol) were taken in H_2O (10 mL) and allowed to stir at room temperature. The reaction was monitored using TLC. After the completion of the reaction, the solid

products **4a**–**h** were collected by filtration. The crude products **4a**–**h** were used for the 2nd step without further purification.

*Step 2 (Synthesis of **5a**–**h**):* The crude product of the 1st step (0.5 mmol) was refluxed in DMF (5 mL). After the completion of the reaction as monitored by TLC, DMF was evaporated out under vacuum. The crude residue was purified by column chromatography using silica gel (100–200 mesh) as adsorbent and Ethyl acetate–Hexane (1:4) as eluent.

14. *General procedure for the reduction of –NO_2 to –NH_2 via Scheme 6:* The nitro compound was dissolved in acetone (3 mL/mmol) and aqueous NaOH (0.5 N, 5 equiv). Excess sodium hydrosulfite (5 equiv) was added and the reaction was refluxed for 1.5 h. Acetone was evaporated, residue was taken up in ethyl acetate and washed with water, brine and dried over Na_2SO_4. Ethyl acetate was evaporated under vacuum and the residue was purified by column chromatography using silica gel (100–200 mesh) as adsorbent and Ethyl acetate–Hexane (1:3) as eluent.

*6-Amino-1,3,7-trimethyl-5-phenylpyrido[2,3-d]pyrimidine-2,4(1H,3H)-dione (**8a**):* Solid; mp 134 °C; IR (KBr) (ν_{max}/cm^{-1}) 3467, 3369, 2928, 2374, 1702, 1647, 1570, 1455, 131417; δ_H (400 MHz, DMSO-d_6, TMS) 7.05–7.41 (m, 5H), 4.16 (s, b, 2H), 3.50 (s, 3H), 3.05 (s, 3H), 2.41 (s, 3H) ppm; δ_C (100 MHz, $CDCl_3$, TMS) 161.1, 151.2, 149.6, 143.8, 135.9, 129.1, 129.0, 128.1, 127.6, 127.1, 106.5, 29.6, 28.3, 21.7 ppm; MS (ESI) *m*/*z* 296 (M)$^+$; Anal. Calcd (%) for $C_{16}H_{14}N_4O_4$: C, 64.85; H, 5.44; N, 18.91%. Found: C, 64.93; H, 5.52; N, 18.83%.