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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, characterization, and urease inhibition of 5-substituted-8-methyl-2*H*-pyrido[1,2-a]pyrimidine-2, 4(3*H*)-diones

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Abstract 5-Substituted-8-methyl-2*H*-pyrido[1,2-*a*]pyrimidine-2,4(3*H*)-dione and its anilines, amino pyridines and hydrazides derivatives were prepared in a good to excellent yields. In the first step 8-methyl-2*H*-pyrido[1,2-*a*]pyrimidine-2,4(3*H*)-dione (**1**) was prepared by reacting 4-methyl-2-aminopyridine, with diethylmalonate. Compounds substituted pyrido[1,2-a]pyrimidine-2,4(3*H*)-diones (PPMDO) (**2**)–(**17**) were prepared by condensing 8-methyl-2*H*-pyrido[1,2-*a*] pyrimidine-2,4(3*H*)-dione in the presence of triethylorthoformte (TEF) and dimethylformamide (DMF), with respective amino components viz. 2-aminoacetophenone, 3-aminoacetophenone, 4-aminoacetophenone, 2,4,6-trimethylaniline, 2-fluoroaniline, 3-fluoroaniline, 4-fluoroaniline, 2-aminothiophenol, 2-amino-4-methylpyridine, 2-amino-5-methylpyridine, 2-amino-5-nitropyridine, Benzoic hydrazide, 4-nitrobenzoic hydrazide, 4-bromobenzoic hydrazide, 4-chlorobenzoic hydrazide and 4-hydroxybenzoic hydrazide, respectively. The chemical structures of all the compounds were elucidated by IR, ^{1}H-NMR, ^{13}C-NMR and elemental analysis data. The synthesized compounds were screened for their in vitro urease inhibition activity, by the phenol hypochlorite method. These compounds were found to exhibit either no or low to moderate or significant activity. The compounds (**9**) and (**14**) showed comparatively much higher activity. However, the compound (**9**) was found to be the most active one.

Keywords Anti-urease · Pyridopyrimidines · Dimroth rearrangement · Virtual screening

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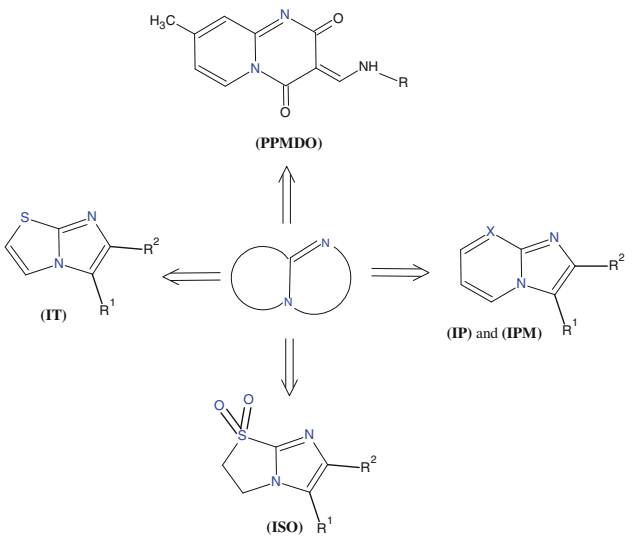
Introduction

Pyridopyrimidine has wide range of biological activity, particularly in cancer and virus research (Berthelot, 1859; Mamouni *et al.*, 2003; David *et al.*, 1997). The pyridopyrimidine is of interest as inhibitor of dihydrofolate reductase (Mihailo *et al.*, 2009) but also for its antibacterial, antifungal (Miller *et al.*, 2009; Sayed *et al.*, 2005), antitumor (Cockerill *et al.*, 2001), adenosine kinase inhibitors (Gfesser *et al.*, 2003; Cowart *et al.*, 2001) activities and recently as excellent potent Akt1/2 Inhibitors (Wu *et al.*, 2008). Furthermore, pathologies associated with the biological system of PDE4 represent some of the more currently important therapeutic targets such as asthma (Finan and Thomas, 2004). Moreover, it has been proved that some of the urea derivatives that have established potent activity over a wide spectrum of tumor cell lines exhibited a good profile for preclinical antitumor investigations (Mounneto *et al.*, 2001).

Keeping in view the historical importance and present prospects of pyridopyrimidines, we were inspired and thus report a novel class of compounds which we prepared, characterized and screened for in vitro urease inhibition. This study is an extension to deal with the synthesis of

8-methyl-2*H*-pyrido[1,2-*a*]pyrimidine-2,4(3*H*)-dione and its aminopyridine, aniline and hydrazide derivatives.

In the past, intensive attention was mainly devoted to the Imidazo[1,2-a]pyridines (IP), Imidazo[1,2-a]pyrimidines (IPM), (Elkakmaoui *et al.*, 1994; Gudmundsson and Johns, 2007; Liang *et al.*, 2007; Scribner *et al.*, 2007; Hayakawa *et al.*, 2007; Warshakoon *et al.*, 2006); Imidazo[1,2-a]thiazoles (IT) and Imidazo[1,2-a]thiazoles (ISO) (Andreani *et al.*, 1995, 1996, 1999, 2000, 2001, 2005) bioactivity (Scheme 1). However, from a chemical point of view, a second isomeric structure is possible. For the development of binding approaches for (IPM) and its analogs (**2**)–(**17**) in the environment, the identification of the active pyrido[1,2-a] pyrimidine-2,4(3*H*)-diones (PPMDO) structures present is important. Neither experimental nor theoretical data is available for the identification of water-solved (PPMDO) species. Theoretically, NMR spectroscopy could be useful for identifying chemical structures. Theoretical ab initio studies could supplement these measurements. In addition, calculations of energetics, atomic charges, minimum energy structures, geometry, and partial π-charge of heteroatoms (Petra) could indicate the electronic density distribution of each atom. Finally, by taking Petra results showing the presence of $N^{\delta+}$=C–$N^{\delta-}$ dipolar system in consideration, realistic Lewis structures can be determined. These systematic data, regarding the variation of molecular properties, are important for the chemical structure and could therefore provide first insights into the still poorly understood chemical opening/closing pyridine or pyrimidine of (IP), (IPM), and (PPMDO) systems.



Scheme 1 Molecules subjected to possible Dimroth opening/closing heterocyclic rings (X = N,CH). (IP): Imidazo[1,2-a]pyridines (X = CH); (IPM): Imidazo[1,2-a]pyrimidines (X = N); (IT): Imidazo[1,2-a]-thiazoles (X = S); (ISO): Imidazo[1,2-a]thiazoles (X = N). (PPMDO): pyrido[1,2-a]pyrimidine-2,4(3*H*)-diones

The results provide an unprecedented opportunity for detailed comparison of 2/3/4-aminoacetophenones, 2,4,6-trimethylaniline, 2/3/4-fluoroanilines, 2-aminothiophenol, 2-amino-4/5-methylpyridines, 2-amino-5-nitropyridine and 4-Cl/Br/OH/NO_2-benzoic hydrazides, functionalization in position N of amino group (–NHR) of the same pharmacophore (PPMDO).

In brief, the objective of this study is to investigate the potential pharmacophore sites of (PPMDO) species using anti-urease screening dependence on pH and comparison with the calculated molecular properties. To verify these structures, further Osiris/Molinspiration (OM) analyses were carried out for example calculation of toxicity risks, solubility, drug-likeness, drug-scor, and lipophicity. Finally, to investigate the anti-urease bioactivity of the (PPMDO) species, tautomeric structure were performed.

Chemistry

Synthesis of compounds (**1**)–(**17**)

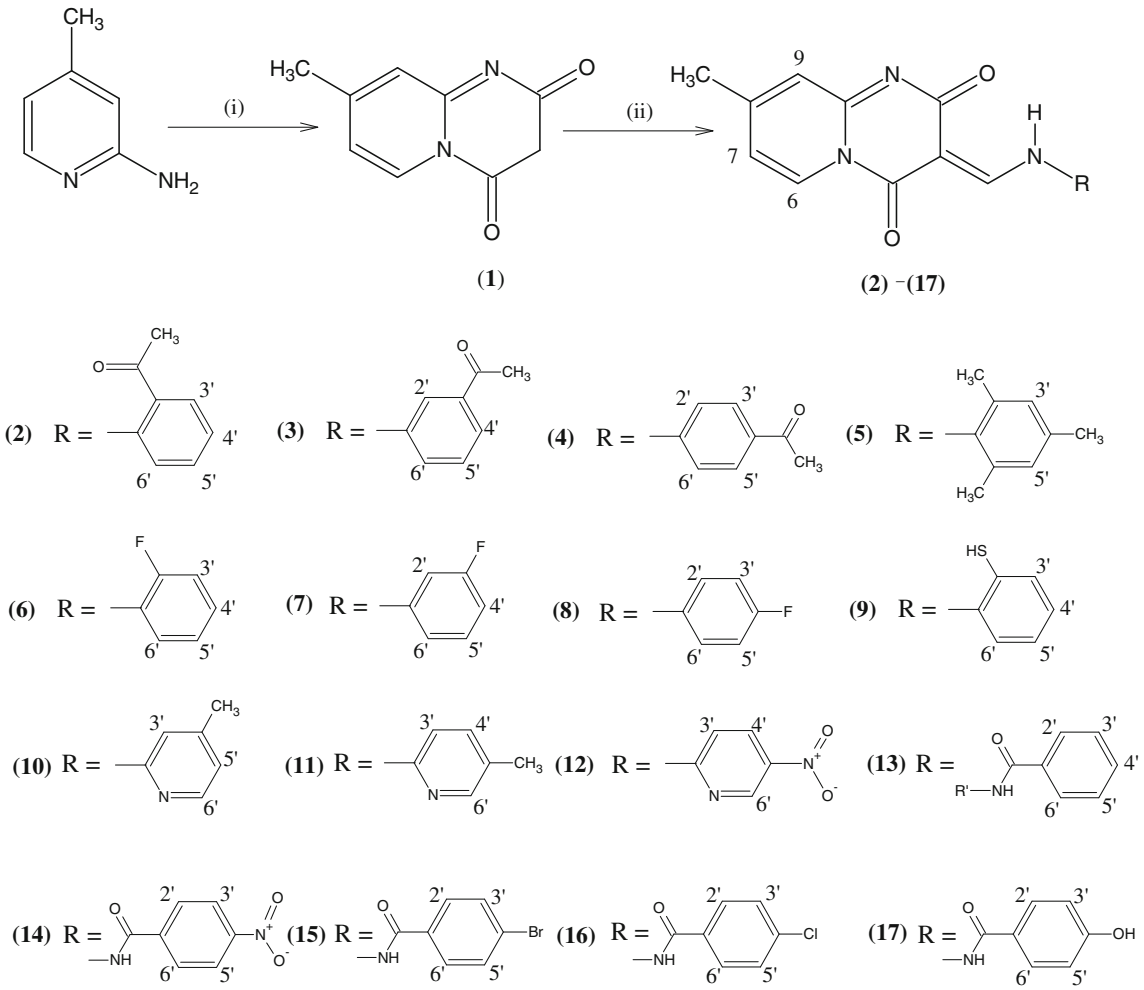
Three methods were adopted for the synthesis of 8-methyl-2*H*-Pyrido[1,2-a]pyrimidine-2,4(3*H*)dione (**1**). In the first method, the reaction of 4-methyl-2-aminopyridine and diethylmalonate was carried out in 2-butanol (as a solvent) and catalytic amount of glacial acetic acid. The product was collected in low yield, i.e., 28% in pure state. In the second method, the same reaction of 4-methyl-2-amino-pyridine and diethylmalonate was carried out in glacial acetic acid (as solvent). The product in this reaction was obtained in 25% yield. However, in the third method, the reaction of 4-methyl-2-amino pyridine and diethylmalonate was carried out without solvent. The product in this reaction was obtained in 72% yield. Direct condensation of 4-methyl-2-aminopyridine with diethyl malonate gave product in good yield (Scheme 2).

Compounds (**2**)–(**17**) were prepared by refluxing equimolar quantities of 8-methyl-2*H*-Pyrido[1,2-a] pyrimidine-2,4(3*H*)dione (**1**) with respective anilines, aminopyridines, or hydrazides in the presence of triethylorthoformate (Scheme 2). The structures of all the synthesized compounds were established through ^{1}H-NMR and elemental analysis data, and ^{13}C NMR were recorded for compounds (**2**), (**10**), and (**13**).

Pharmacology

Biological activity: urease inhibition (in vitro)

Compounds (**1**)–(**17**) were tested for their urease inhibition potential in concentration range from 100 μM to 0.01 μM



Scheme 2 Synthesis of compounds (**1**)–(**17**). (i) Diethylmalonate/reflux (4 h); (ii) R-NH_2/Ethylorthoformate/Dimethylformamide/reflux (4 h)

(Table 1). Compound (**9**) showed the highest inhibitory potential at the 100 μM concentration followed by compounds **9** > **14** > **2**>**15** > **11** > **6**>**12** > **3**>**16** > (**1** = **17**) ≥ **8**≥ **10** > **7**>**13** > **4**. The extent inhibition was concentration-dependent. Compound (**5**) at all the concentrations tested and compounds (**8**), (**10**)**,** (**15**), and (**16**) at some lower concentrations did not inhibit activity of the enzyme. This effect could be attributed to the lower effective concentration of the test compound and to the nature of the test compound and the corresponding functional groups present therein (see below). These observations suggest that compounds (**9**) and (**14**) are strong inhibitors; compounds (**2**), (**6**), (**11**), and (**15**) are moderate urease inhibitors; while compounds (**1**), (**3**), (**4**), (**7**), (**8**), (**10**), (**12**), (**13**), (**16**), and (**17**) are weak inhibitors of the urease enzyme.

Effect of various substitutions can be assessed by comparing the inhibitory characteristic of the derived compounds with that of the compound (**1**), the starting material which belongs to the weak urease inhibitor group of molecules. Compounds (**9**), (**14**), (**2**), (**15**), (**11**), (**6**), (**12**), (**3**), and (**16**) are more potent inhibitors than the starting material whereas the compounds (**17**), (**8**), (**10**), (**7**), (**13**), and (**4**) are less potent inhibitors. These derivatives were synthesized by treating 8-methyl-2*H*-pyrido[1,2-*a*] pyrimidine-2,4(3*H*)-dione (**1**) with 2-aminothiophenol, 4-nitobenzoichydrazide, 2-aminoacteophenone, 4-bromobenzoichydrazide, 2-amino-5-methylpyridine, 2-fluoroaniline, 2-amino-5-nitropyridine, 3-aminoacetophenone, 4-chlorobenzoichydrazide, 4-hydroxybenzoic hydrazide, 4-fluoroaniline, 2-amino-4-methyl-pyridine, 3-fluoroaniline, benzoichydrazide, and 4-aminoacetophenone, respectively.

Since Urease is a metallo-enzyme requiring two Ni^{+2} ions for its activity the presence of a metal chelating group like the –SH group [compound (**9**)] would result in significant inhibition of the enzyme activity whereas the absence of such a group or the introduction of a non-chelator group would tend to decrease the metal chelating

Table 1 Urease inhibition of 8-methyl-2*H*-pyrido[1,2-*a*]pyrimidine-2,4(3*H*)-dione (**1**) and functionalized derivatives (**2**)–(**17**)

Compound	% Inhibition in concentration range from 100 to 0.01 μM				
	100 μM	10 μM	1 μM	0.1 μM	0.01 μM
1	39	26	12	6	1
2	69	54	32	19	11
3	45	28	8	3	−1
4	18	5	1	−2	−3
5	0.2	−8	−1	−6	−16
6	57	44	26	10	3
7	28	3	−25	−30	−25
8	35	21	14	6	1
9	97	73	60	28	22
10	33	20	16	6	1
11	57	48	24	11	3
12	48	36	23	6	3
13	26	13	6	1	−1
14	87	60	54	21	6
15	57	23	3	−2	−8
16	43	14	6	−2	−4
17	39	22	7	−1	−5
Thiourea	94	21	15	8	8

property of the compound being synthesized. Reaction with 2,4,6-trimethylaniline did not modify the base line urease inhibition activity of the starting material.

Results and discussion

Spectroscopic characterization of compounds (**1**)–(**17**)

IR spectra

The IR spectra of (**2**)–(**17**) also help to confirm the coexistence, in solution, of their tautomeric structures by showing, example for compound (**2**), two bands at 3211/3060 cm^{-1} for aminic N–H group of (-RN-H), two bands at 1723/1685 cm^{-1} for carbonyl C = O stretching. The IR data of (**2**) support the absence of hydrogen on oxygen atoms of two carbonyl groups by showing no O–H absorbance at 3400–3450 cm^{-1}, which confirms the idea that some tautomeric forms are favored than others (Table 2). Interestingly we observe, in selected ^{1}H NRM data (Table 1), the coexistence of Z and E isomers controlled by Dimroth process (Table 2).

^{1}H NMR spectra

The ^{1}H NMR spectra of all the compounds (**2**)–(**17**), pyrimidine-2,4-dione *C*-7 protons appeared as doublet at δ 7.32–7.43. The *C*-9 protons experienced deshielding due to the only inductive effect of the (=N–C=O) and were recorded downfield as singlet at δ 7.42–7.59 as compared to *C*-7 protons, which are experiencing less deshielding by virtue of its position and nature of bonding. The *C*-6 protons being deshielded due to the inductive effect of (–N–C=O) functionality and on the other side electron withdrawing effect of the (=N–C=O) functionality of the pyrimidine-2,4-dione, appeared further downfield as double doublet at δ 7.70–7.85. In all compounds, a very little difference in the chemical shifts of *C*-6 protons, *C*-7 protons, and *C*-9 protons was observed, which was due to the very small inductively electron withdrawing or donating effect of substituted anilino or pyridinyl or benzohydrazide functionalities, which are far away from pyrimidine-2,4-dione ring. Similarly a very little difference in the chemical shifts of methyl protons present on the pyrimidine-2,4-dione ring was observed and appeared as singlet at δ 2.18–2.27.

The ^{1}H NMR spectrum of compound (**2**) demonstrated *C*-3′ proton, ortho to acetyl and *meta* to amino group experiencing deshielding due to resonance and inductive effect of acetyl group appeared downfield as double doublet at δ 7.50. The *C*-5′ proton para to acetyl and *meta* to amino group experiencing deshielding due to electron withdrawing effect of acetyl group but experiencing less inductive effect by virtue of its position, resonated slightly upfield as double of double doublet at δ 7.35. Whereas *C*-6′ proton appeared upfield as compared to *C*-5′ proton as double doublet because of the shielding effect of the amino functionality. The acetylanilino *C*-4′ proton appeared further upfield as compared to *C*-5′ proton as double of double doublet at δ 7.20, being shielded due to the resonance effect of amino group and experiencing less inductive effect due to acetyl and amino functionalities by virtue of its position.

The ^{1}H NMR spectrum of compound (**3**) demonstrated *C*-2′ proton, *ortho* to acetyl and amino groups experiencing deshielding due to electron withdrawing nature of acetyl functionality, however, being shielded by the electron donating effect of amino group, appeared as singlet at δ 7.69. The *C*-4′ proton *ortho* to acetyl and para to N–H functions experiencing deshielding due to electron attracting effect of acetyl functional group, however, being shielded by the electron donating effect of amino functionality but experiencing less inductive effect by virtue of its position, resonated slightly upfield as double doublet at δ 7.58. The acetylanilino *C*-6′, experiencing a deshielding effect due to the electron withdrawing effect of acetyl group, however, being shielded due to the electron donating effect of amino group. This in turn experiences less inductive effect by these two functional groups and appeared upfield as double doublet at δ 7.36. The *C*-5′ proton *meta* to acetyl and NH functions appeared further

Table 2 Selected IR and ^{1}H NMR data of compounds (**2**)–(**17**)

Compd.	Selected IR data (υ, cm^{-1})				Selected ^{1}H NMR data	
	(–RN–H)^1	(–RN–H)^2	(C=O)^1	(C=O)^2	(=C***H***–N)^1, (=C***H***–N)^2	(=C–N***H***)^1, (=C–N***H***)^2
2	3291 (m)	3098 (m)	1723 (s)	1685 (m)	9.10 (1H, d, *J* 12.7)	12.32 (1H, d, *J* 13.4)
					9.32 (1H, d, *J* 13.6)	13.25 (1H, d, *J* 12.7)
3	3211 (m)	3060 (m)	1700 (s)	1645(m)	9.0 (1H, d, *J* 12.9)	12.90 (1H, d, *J* = 13.3)
					9.20 (1H, d, *J* 13.4)	13.18 (1H, d, *J* 12.9)
4	3209 (m)	3060 (m)	1703 (s)	1645 (m)	8.98 (1H, d, *J* 13.0)	12.92 (1H, d, *J* 13.2)
					9.22 (1H, d, *J* 13.6)	13.22 (1H, d, *J* 13.0)
5	3279 (m)	3080 (m)	1700 (s)	1685 (m)	8.88 (1H, d, *J* 13.5)	11.32 (1H, d, *J* 13.1)
					9.15 (1H, d, *J* 13.3)	12.45 (1H, d, *J* 13.5)
6	3211 (m)	3029 (m)	1709 (s)	1675 (m)	8.92 (1H, d, *J* 13.4)	11.45 (1H, d, *J* 13.6)
					9.20 (1H, d, *J* 13.8)	12.88 (1H, d, *J* 13.4)
7	3259 (m)	3064 (m)	1710 (s)	1645 (m)	8.83 (1H, d, *J* 13.5)	11.50 (1H, d, *J* 13.3)
					9.10 (1H, d, *J* 13.4)	12.80 (1H, d, *J* 13.5)
8	3260 (m)	3042 (m)	1725 (s)	1640 (m)	8.75 (1H, d, *J* 13.2)	11.44 (1H, d, *J* 13.0)
					9.0 (1H, d, *J* 13.5)	12.76 (1H, d, *J* 13.2)
9	3270 (m)	3007 (m)	1716 (s)	1660 (m)	9.62 (1H, d, *J* 13.8)	11.22 (1H, d, *J* 13.2)
					9.90 (1H, d, *J* 13.5)	12.35 (1H, d, *J* 13.8)
10	3288 (m)	3010 (m)	1706 (s)	1680 (m)	8.72 (1H, d, *J* 12.8)	12.21 (1H, d, *J* 12.9)
					9.32 (1H, d, *J* 13.1)	13.42 (1H, d, *J* 12.8)
11	3269 (m)	3010 (m)	1730 (s)	1685 (m)	8.82 (1H, d, *J* 12.3)	12.19 (1H, d, *J* 12.7)
					9.30 (1H, d, *J* 12.9)	13.41 (1H, d, *J* 12.3)
12	3267 (m)	3034 (m)	1706 (s)	1665(m)	8.91 (1H, d, *J* 12.3)	12.72 (1H, d, *J* 12.9)
					9.45 (1H, d, *J* 13.2)	13.68 (1H, d, *J* 12.3)
13	3275 (m)	3021 (m)	1718 (s)	1651 (m)	8.45 (1H, d, *J* 13.4)	11.35 (1H, d, *J* 13.8)
					8.75 (1H, d, *J* 13.2)	11.96 (1H, s)
						12.45 (1H, d, *J* 13.4)
14	3235 (m)	3010 (m)	1720 (s)	1685 (m)	8.82 (1H, d, *J* 12.2)	12.0 (1H, s)
					9.45 (1H, d, *J* 12.8)	12.55 (1H, d, *J* 12.5)
						13.40 (1H, d, *J* 12.2)
15	3290 (m)	3010 (m)	1735 (s)	1670 (m)	8.56 (1H, d, *J* 13.7) 8.85 (1H, d, *J* 14.2)	11.40 (1H, d, *J* 13.9)
						11.93 (1H, s)
						12.65 (1H, d, *J* 13.7)
16	3210 (m)	3011 (m)	1718 (s)	1666 (m)	8.60 (1H, d, *J* 13.6) 8.90 (1H, d, *J* 13.9)	11.50 (1H, d, *J* 13.7)
						11.94 (1H, s)
						12.67 (1H, d, *J* 13.6)
17	3275 (m)	3013 (m)	1710 (s)	1665 (m)	8.44 (1H, d, *J* 13.8) 8.83 (1H, d, *J* 14.2)	11.42 (1H, d, *J* 14.0)
						11.88 (1H, s)
						12.60 (1H, d, *J* 13.8)

upfield as compared to *C*-6′ proton as double doublet at δ 7.23, being not shielded or deshielded by both functionalities and experiencing only inductive effect (-I) due to acetyl and amino group by virtue of its position.

The ^{1}H NMR spectrum of compound (**4**) verified acetylanilino *C*-3′ and *C*-5′ protons are equivalent protons, *ortho* to acetyl and *meta* to amino functionalities experiencing deshielding due to resonance and inductive effect of acetyl functionality, appeared downfield as triplet at δ 7.62. The *C*-2′, *C*-6′ protons are magnetically equivalent and *ortho* to amino and *meta* to acetyl group and being shielded due to electron donating effect of amino group and resonated upfield as triplet at δ 7.32. In all the three compounds (**2**–**4**), acetyl methyl protons appeared at δ 3.21–3.24.

The ^{1}H NMR spectrum of compound (**5**) displayed three methyl protons present on the anilino ring para to amino functionality as singlet at δ 2.16; however, six methyl protons *ortho* to amino group appeared slightly downfield

at δ 2.18. In both cases electron donating effect due to amino functionality is same but *ortho* methyl groups experiencing more inductive effect as compared to para methyl group due to amino group. As far as the exhibition of *C*-3′, *C*-5′ equivalent protons is concerned, experiencing no shielding or deshielding due to amino group, however, being shielded by the inductively electron donating effect of methyls and appeared as singlet at δ 7.0.

The ^{1}H-NMR spectrum of compound (**6**) confirmed *C*-3′ proton, *ortho* to fluoro and *meta* to amino group experiencing deshielding due to the inductive effect of fluorine, appeared downfield as double doublet at δ 7.30. The *C*-5′ proton para to fluoro and *meta* to amino group experiencing less inductive effect by virtue of its position, resonated slightly upfield as double of double doublet at δ 7.24. As far as the exhibition of *C*-6′ proton is concerned, it was recorded upfield as compared to *C*-5′ proton as double doublet at δ 7.18 because of the shielding effect of the amino functionality. The fluoroanilino *C*-4′ proton appeared further upfield as compared to *C*-6′ proton as double of double doublet at δ 7.10, being shielded due to the resonance effect of amino functionality and experiencing less inductive effect due to fluoro and amino functionalities by virtue of its position.

The ^{1}H NMR spectrum of compound (**7**) demonstrated *C*-5′ proton *meta* to fluoro and amino functionalities, being not shielded by both functional groups and experiencing only inductive effect (-I) due to these groups appeared downfield as double doublet at δ 7.34. The fluoroanilino *C*-2′ proton, *ortho* to fluoro and amino groups experiencing deshielding due to inductively electron withdrawing nature of fluoro function, however, being shielded by the electron donating effect of amino group, appeared upfield as singlet at δ 7.27. The *C*-4′ proton *ortho* to fluoro and para to amino functionalities experiencing deshielding due to inductively electron withdrawing effect of fluorine, however, being shielded by the electron donating effect of amino group but experiencing less inductive effect as compared to *C*-2′ proton by virtue of its position, resonated slightly upfield as double doublet at δ 7.19. The fluoroanilino *C*-6′ proton, being shielded due to the electron donating effect of amino functionality, which in turn is experiencing less inductive effect by these two functional groups appeared further upfield as double doublet at δ 7.12.

The ^{1}H NMR spectrum of compound (**8**) verified fluoroanilino *C*-3′ *C*-5′ equivalent protons, *ortho* to fluoro and *meta* to amino functionalities experiencing deshielding inductive effect of fluorine, appeared downfield as doublet at δ 7.25. The *C*-2′ *C*-6′ proton *ortho* to amino and *meta* to fluoro group being shielded due to electron donating effect of amino group, resonated upfield as doublet at δ 7.11. Which was due to the difference in the inductively electron withdrawing influence of the fluorine atom present on the anilino ring.

The ^{1}H NMR spectrum of compound (**9**) demonstrated sulfanyl aniline *C*-3′, *C*-4′, *C*-5′, and *C*-6′ protons appeared as multiplet at δ 7.10–7.22. As for as the exposition of the sulfanyl (S–H) group is concerned, appeared as singlet at δ 4.22.

The ^{1}H NMR spectrum of compound (**10**) demonstrated *C*-6′ proton, being deshielded by strong electron withdrawing effect of pyridinyl nitrogen both by resonance and inductive effect, appeared downfield as double at δ 8.20. The pyridinyl *C*-3′ proton again deshielded by strong electron withdrawing effect of pyridinyl nitrogen by resonance and experiencing less inductive effect as compared to *C*-6′ proton, appeared slightly upfield as singlet at δ 7.88. As for as the display of *C*-5′ proton is concerned it experiencing deshielding due to only inductive effect of nitrogen by virtue of its position, appeared further upfield as singlet at δ 7.48. The methyl protons present on the pyridinyl ring appeared as singlet at δ 2.30.

The ^{1}H NMR spectrum of compound (**11**) confirmed *C*-6′ proton, being deshielded by strong electron withdrawing effect of pyridinyl nitrogen both by resonance and inductive effect, appeared as singlet at δ 8.10, slightly upfield in comparison with compound (**10**) due to the position of methyl group on the ring. The pyridinyl *C*-4′ proton again deshielded by strong electron withdrawing effect of pyridinyl nitrogen by resonance and experiencing less inductive effect as compared to *C*-6′ proton, appeared slightly upfield as singlet at δ 7.96. As for as the display of *C*-3′ proton is concerned, it experiencing deshielding only, due to inductive effect of the nitrogen, appeared further upfield as singlet at δ 7.48. The methyl protons present on the pyridinyl ring appeared, slightly downfield as because of closeness to pyridinyl nitrogen compared to compound (**10**) as singlet at δ 2.32.

The ^{1}H NMR spectrum of compound (**12**) demonstrated *C*-6′ proton, being deshielded by strong electron withdrawing effect of pyridinyl nitrogen and nitro functional groups by resonance effect, as well as by strong inductive effect, appeared much downfield as singlet at δ 9.21. The pyridinyl *C*-4′ proton, again deshielded by strong electron withdrawing effect of pyridinyl nitrogen and nitro group by resonance and experiencing less inductive effect as compared to *C*-6′ proton, appeared as doublet at δ 8.88. As for as the exhibition *C*-3′ proton is concerned, it experiencing deshielding due to only inductive effect of the nitrogen as well as nitro groups, appeared as doublet at δ 8.20. The methyl protons present on the pyridinyl ring appeared, slightly downfield as because of closeness to pyridinyl nitrogen‘ compared to compound (**10**) as singlet at δ 2.32.

The ^{1}H NMR spectrum of compound (**13**) verified benzohydrazide *C*-2′, *C*-6′ equivalent protons, *ortho* to (–NH–C=O) functionality experiencing deshielding due to resonance and inductive effect of that functionality,

appeared downfield as doublet at δ 7.88. The *C*-4′ proton para to same functional group experiencing deshielding due to resonance effect but less inductive effect by virtue of its position appeared as triplet at δ 7.51. The *C*-3′, *C*-5′ proton *meta* to –NH–C=O functional group being not deshielded due to electron withdrawing effect of same group resonated upfield as doublet at δ 7.37. The ^{1}H NMR spectra of compounds (**15**)–(**17**) demonstrated benzohydrazide *C*-2′, *C*-6′ equivalent protons, *ortho* to –NH–C=O and *meta* to –Br, Cl, and OH functional groups, experiencing deshielding due to resonance and inductive effect of amide function, appeared as doublet at δ 7.90–7.94. The *C*-3′, *C*-5′ protons *meta* to –NH–C = O functionality being not deshielded due to electron withdrawing effect of amide group but experiencing shielding due electron donating effect of Br, Cl, and OH functional groups, resonated upfield as doublet at δ 7.39–7.45. The –N–NH protons appeared as singlet at δ 11.92–11.94. In addition, In the case of compound (**17**), hydroxylic (OH) proton was appeared as singlet at δ 9.75.

The ^{1}H NMR spectrum of compound (**14**) demonstrated benzohydrazide *C*-2′, *C*-6′ equivalent protons, *ortho* to –NH–C=O and *meta* to nitro (–NO_2) groups, experiencing deshielding due to electron withdrawing nature of amide and inductively (-I) of amide and nitro groups, appeared as doublet at δ 8.21. The *C*-3′, *C*-5′ proton *meta* to –NH–C=O and *ortho* to nitro groups being deshielded more due to strong electron withdrawing effect of the nitro group both by resonance and inductive effects, displayed further downfield as doublet at δ 8.32. Similarly, –N–NH proton appeared downfield as singlet at δ 12.0 as compared to compounds (**15**)–(**17**).

The ^{1}H NMR spectra of compounds (**2**)–(**17**) exhibited split doublets between δ 11.22–12.92 and δ 12.35–13.42 and between δ 8.44–9.10 and δ 8.75–9.90 ascribed to =C–NH and (=CH–N) protons, respectively. The splitting was due to the existence of Z and E isomers (Hamdi *et al.,* 1992; Skaellariou *et al.,* 1990). ^{13}C NMR of reference compounds is also reported. The elemental analysis data agree well with calculated values.

^{13}C NMR spectra

^{13}C NMR spectra of (**2**–**17**) were recorded; however, (**2**), (**10**), and (**13**) which are representative compounds of each series are discussed here and found to be consistent with the proposed structures at each instance. Compound (**2**) clearly gave three carbonyl carbons at δ 158.8, 169.4 and 201.7, the first two accounts for the carbonyl at the ring and last one markedly upfield due to the acetyl carbonyl carbon. There were eight quaternary and eight methine (CH) carbons were traced in total, which justified the structure suggested. Two methyl peaks were also evident at 24.1 and 31.3; again the upfield signal was due to acetyl methyl group whereas downfield was due to methyl group attached with the ring. ^{13}C NMR of (**10**) recorded fifteen signals altogether, δ 167.1 and 164.2 accounted for the two amide carbonyls and there were four more quaternary carbons as well recorded at δ 155.4, 148.3, 147.1, and 107.7. Two methyl groups were observed at δ 23.0 and 22.6 along with seven methine carbons which accounted for the methine aromatic carbons and vinylic carbon, respectively. Similarly, ^{13}C NMR of (**13**) was in good agreement with the proposed structure as it displayed three carbonyls of the amides at δ 174.2, 160.7, and 153.5. Three quaternary carbons observed at δ 143.8, 136.3, and 107.3 along with nine methine carbons which represented aromatic and vinylic carbons. A signal at 20.5 accounted for the methyl group present at the aromatic ring. All the ^{13}C NMR data effectively favored the proposed structures which was complemented with ^{1}H NMR and elemental analysis records.

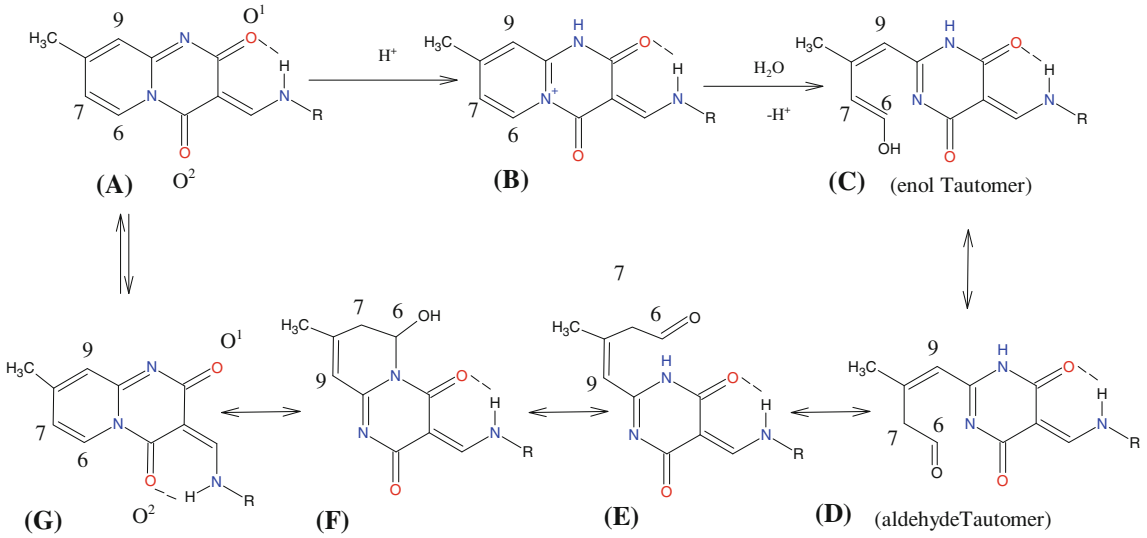
Molecular properties calculations

Tautomerism of (PPMDO) compounds (2)–(17)

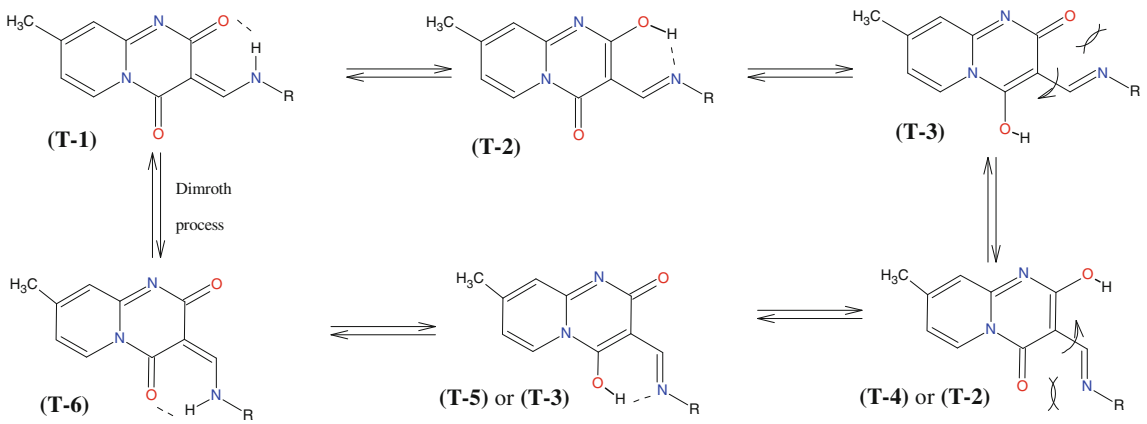
For substituted Imidazo[1,2-a]pyridine (-pyimidine) (IP) and (IPM) and certainly for their analogs (**2**)–(**17**), depending on the pH, two major possible (PPMDO) conformations can be described for the neutral form. This isomerism is governed by the mechanism of Dimroth process. These relevant structures are sketched in Scheme 3.

On the other hand, the two isomeric forms (**A**) and (**G**), depending on the pH, four principal possible tautomeric limit forms can be described for the neutral parent form. This tautomerism is governed by the bond dissociation energies of bonds (O–HN and OH–N=C), sigma charge distribution, π-charge distribution of aromatic bicyclic system (PPMDO), inductive effect of substituent R of amine (–NH–R), resonance effect and delocalization energies and polarizability effect. So the partial π-charge of the two oxygen atoms of carbonyl groups and the electronic nature of substituent R of amine (–NH–R) are strongly involved in the leading to the most stable tautomer. These relevant structures are sketched in (Scheme 4).

Current thinking in the generation of specific drug leads embodies the concept of achieving high molecular diversity within the boundaries of reasonable drug-like properties (Koehn and Carter, 2005). Natural and semi-natural products have high chemical diversity, biochemical specificity and other molecular properties that make them favorable as lead and standard reference (SR) structures for drug discovery, and which serve to differentiate them from libraries of synthetic and combinatorial compounds. Various investigators have used computational methods to



Scheme 3 Dimroth process: opening/closing pyridine ring



Scheme 4 Phenomenon of tautomerism

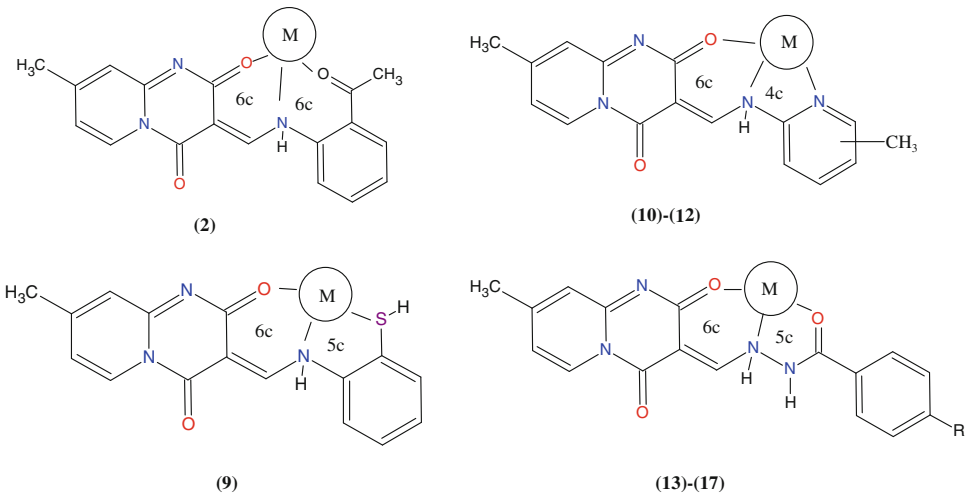
understand differences between natural products and other sources of drug leads (Carroll *et al.*, 2006). Modern drug discovery is based in large part on high throughput screening of small molecules against macromolecular disease targets requiring that molecular screening libraries contain drug-like or lead-like compounds. We have analyzed known standard references (SR) for drug-like and lead-like properties. With this information in hand, we have established a strategy to design specific drug-like or lead-like (PPMDO) (**2**)–(**17**).

The series (**2**)–(**17**) have been subjected to tautomerization and charge-delocalization using ACD method of the amino-hydrogen atom (Scheme 5), obtained from the proton-transfer of the amino group (-NHR), have been used to model the bioactivity against urease. We give here, as example, the compounds (**2**), (**9**), (**10**–**12**), and (**13**–**17**).

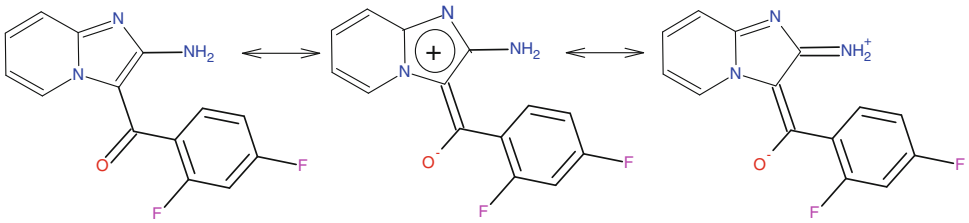
It is found that the ligands containing 5/6 bonded-coordination pseudo-cycles of (**9**) contribute positively in favor of an anti-urease activity, more, and this is in good agreement with the mode of thermodynamic stabilité of metal complexes [(**5c**,**5c**) > (**5c**,**6c**) > (**4c**,**6c**) > (**6c**,**6c**)] according angle of Bits. It was hypothesized that potential Nickel chelatation of ligands may facilitate the inhibition of urease, more than non-chelator ligands. It is further found that the activity increases with increase in negative charge of heteroatoms of the common pharmacophore fragment of the potential tautomers (**T-1** and **T-6**). The presence of conformation/tautomerism phenomena in aza-bicyclic analogs was presented previously since 1999, in few articles by some rare authors (Hamdouchi *et al.*, 1999; Jaramillo *et al.*, 2006) (Scheme 6).

The structural parameters indicate a stereodynamics of Ar–C=O rotation and conformational preferences of 2-amino-3-(2,4-difluorobenzoyl)-imidazo[1,2-a]pyridine (Jaramillo *et al.*, 2006). The main differences between the three tautomeric forms lie in the intramolecular hydrogen

Scheme 5 Potential mode of metal-coordination of compounds (**2**), (**9**), (**10–12**), and (**13–17**), leading ligands to be organized in situ as different tridentate sites of coordination (O,N,O) for (**2**) and (**13**)–(**17**); (O,N,S) for (**9**); and (O,N,N) for (**10**)–(**12**)



Scheme 6 Conformation/ tautomerism phenomena in aza-bicyclic analogs



bonding and the relative orientation of the carbonyl group. Attractive intermolecular interactions occur and are responsible for the value of diahedral angle (C2–C3–C=O). This has a direct impact on bioactivity of compounds (Hamdouchi *et al.*, 1999; Scheme 5). Potential mode of metal-coordination of compounds (**2**), (**9**), (**10–12**), and (**13–17**), leading ligands to be organized in situ as different tridentate sites of coordination (O,N,O) for (**2**) and (**13**)–(**17**); (O,N,S) for (**9**); and (O,N,N) for (**10**)–(**12**).

Osiris calculations[1]

Structure-based design is now fairly routine but many potential drugs fail to reach the clinic because of ADME-Tox liabilities. One very important class of enzymes, responsible for many ADMET problems, is the cyto-chromes P450. Inhibition of these or production of unwanted metabolites can result in many adverse drug reactions. Of the most important program, Osiris is already available online.

With our recent publication of the drug design combination of various pharmacophore sites by using spiro-heterocyclic structure, it is now possible to predict activity and/or inhibition with increasing success in two targets (bacteria and HIV virus). This is done using a combined electronic/structure docking procedure and an example will be given here. The remarkably well behaved mutagenicity of divers synthetic molecules classified in data base of CELERON Compagny of Swiss can be used to quantify the role played by various organic groups in promoting or interfering with the way a drug can associate with DNA. Toxicity risks (mutagenicity, tumorogenicity, irritation, and reproduction) and physic-chemical properties (ClogP, solubility, drug-likness, and drug-scor) of tautomers of compound (**9**) are calculated by the methodology developed by Molinspiration as a sum of fragment-based contributions and correction factors (Table 3).

Molinspiration calculations

CLogP (octanol/water partition coefficient) is calculated by the methodology developed by Molinspiration (Ertl *et al.*, 2000) as a sum of fragment-based contributions and correction factors (Table 4).

The method is very robust and is able to process practically all organic, and most organometallic molecules. Molecular Polar Surface Area TPSA is calculated based on the methodology published by Ertl *et al.* as a sum of

[1] The OSIRIS Property Explorer shown in this page is an integral part of Actelion's inhouse substance registration system. It lets you draw chemical structures and calculates on-the-fly various drug-relevant properties whenever a structure is valid. Prediction results are valued and color coded. Properties with high risks of undesired effects like mutagenicity or a poor intestinal absorption are shown in red. Whereas a green color indicates drug-conform behavior. http://www.organic-chemistry.org/prog/peo/.

Table 3 Osiris calculations of tautomers of compound (**9**)

Tautomer	Toxicity risks				Osiris calculations				
	MUT	TUMO	IRRI	REP	MW	CLP	S	D-L	D-S
T-1	*	*	*	*	311	1.20	−3.75	1.48	0.76
T-2	*	*	*	*	311	0.73	−3.33	1.84	0.81
T-6	*	*	*	*	311	1.20	−3.75	1.48	0.76
T3	*	*	*	*	311	0.96	−4.69	1.63	0.68

* Within suitable limits for drugs

Table 4 Molinspiration calculations of tautomers of compound (**9**)

Tautomer	Molinspiration calculations						Drug-likeness			
	MW	CLP	TPSA	OH–NH	NV	VOL	GPCRL	ICM	KI	NRL
T-1	311	2.48	63	1	0	265	−1.25	−1.52	−0.90	−1.75
T-2	313	2.30	67	2	0	271	−1.12	−0.66	−1.36	−1.61
T-6	311	2.48	63	1	0	265	−1.25	−1.52	−0.90	−1.75
T-3	313	2.08	67	1	0	271	−0.90	−1.07	−1.41	−1.22

fragment contributions. O- and N-centered polar fragments are considered. PSA has been shown to be a very good descriptor characterizing drug absorption, including intestinal absorption, bioavailability, Caco-2 permeability, and blood–brain barrier penetration. Prediction results of compound (**9**) molecular properties (TPSA, GPCR ligand, and ICM) are valued (Table 4).

Conclusions

Of the synthesized compounds (**2**)–(**17**), two compounds (**9**) and (**14**) were found to be significantly more potent inhibitors of urease activity than the starting compound (**1**). The same two compounds also exhibited anti-urease activity comparable to thiourea, a standard urease inhibitor used in this study as a reference. While at 100 μM concentration thiourea reduces activity of the urease enzyme by a factor of 94%, compounds (**9**) and (**14**) reduce activity of this enzyme by a factor of 96 and 86%, respectively. The compounds are potent inhibitors of the urease enzyme even at lower concentrations. While at 0.01 μM concentration the standard urease inhibitor thiourea reduces enzyme activity by a factor of 8% only, compound (**9**) at the same concentration reduces enzyme activity by a factor of 22%. The study being submitted suggests that of all the compounds synthesized during the present investigation, those bearing sulfanylanilino and 4-nitrobenzohydrazide moieties possess significant urease inhibition activity. These compounds may have more tendencies to chelate with the nickel ions required for the activity of the enzyme.

Experimental protocols

Materials and methods

All reagents and solvents were used as obtained from the supplier or recrystallized or redistilled as necessary. Thin-Layer chromatography was performed using aluminum sheets (Merck) coated with silica gel 60 F_{254}. IR spectra were recorded in the range 4000–6000 nm using an IR Perkin-Elmer Spectrum 1 FTIR spectrophotometer and peaks are reported $_{max}$(neat)/cm^{-1} which refer to the ν_{max} in wave numbers. Proton magnetic resonance spectra were recorded in d_6-DMSO with Bruker AM 300 and AM 400 spectrometers (Rheinstetten–Forchheim, Germany) operating at 300 and 400 MHz, respectively. The ^{13}C NMR spectra were recorded in d_6-DMSO with Bruker AM 100 spectrometer operating at 100 MHz. Tetramethyl-silane was used as an internal standard. Elemental analysis for C, H, N, and S were recorded with Perkin-Elmer 2400 Series II CHN Analyzer. Melting points were recorded on a GallenKamp apparatus and are uncorrected.

Procedure for establishing urease inhibition (in vitro)

Urease inhibition was determined by a combination of the protocol adopted by (Pervez *et al.,* 2009 and Weatherburn, 1967). A clear solution of 5 μL (0.1 mM) of the test sample was incubated along with 25 μL of enzyme (Jack bean urease) and 55 μL of buffer containing 100 mM urea in a well plate at 30°C for 15 min. Urease inhibition was determined by measuring the ammonia production using

the indophenol method and percentage inhibitions were calculated at each instance. Thiourea was employed as the standard.

Synthesis of compounds (**1**)–(**17**)

*Preparation of 8-methyl-2H-pyrido[1,2-a]pyrimidine-2,4(3H)-dione (**1**)*

4-Methyl-2-aminopyridine (10 g, 92 mmol) was dissolved in diethyl malonate (16 g, 92 mmol) and the resultant mixture was then heated under reflux for 4 h. Upon completion, the reaction mixture was kept at room temperature for further 1 h till the precipitates appeared. The precipitates were collected by suction filtration and washed several times with diethyl ether which furnished the light yellow product in 72% yield (Scheme 1). The purity of the prepared compounds was checked by Thin-Layer Chromatography and m.p. 268–270°C (Tschitschibabin, 1924; Stadlbauer *et al.*, 2001).

*General procedure for the synthesis of compounds (**2**)–(**17**)*

To a hot stirred solution of (**1**) (0.5 g, 2.8 mmol) and DMF (7 g, 95 mmol) was added appropriate amount of aniline or aminopyridine or hydrazides followed by the addition of triethylorthoformate (0.42 g, 2.8 mmol). The resultant reaction mixture was heated under reflux for 4 h. After completion of reaction, the volume was reduced to one-third. The reaction mixture was allowed to stand for 1 h at room temperature. The contents were poured in distilled water, and the solid thus obtained was collected by suction filtration in pure state (Scheme 2).

*3-[(2-Acetylanilino)methylidene]-8-methyl-2H-pyrido[1,2-a]-pyrimidine-2,4-dione (**2**)* White powder. Mp = 306–308°C. Yield 80%. IR (ν neat)/cm^{-1}) 3291(m), 3098(m), 1723 (s), 1685(m); ^{1}H NMR (d_6-DMSO, δ, ppm): 2.25 (3H, s, C*H*$_3$-pyrimidine-2,4-dione), 3.22 (3H, s, C*H*$_3$CO–), 7.20 (1H, ddd, *J* 7.9, 7.5, 2.1 Hz, acetylanilino C$_4'$-*H*), 7.26 (1H, dd, *J* 8.1, 2.1 Hz, acetylanilino C$_6'$-*H*), 7.35 (1H, ddd, *J* 7.5, 8.1, 2.2 Hz, acetylanilino C$_5'$-*H*), 7.40 (1H, d, *J* 7.4, pyrimidine-2,4-dione C$_7$-*H*), 7.50 (1H, dd, *J* 7.9, 2.2 Hz, acetylanilino C$_3'$-*H*), 7.55 (1H, s, pyrimidine-2,4-dione C$_9$-*H*), 7.82 (1H, d, *J* 7.4 Hz, pyrimidine-2,4-dione C$_6$-*H*), 9.10 (1H, d, *J* 12.7, =C*H*–N–), 9.32 (1H, d, *J* 13.6, =C*H*–N–), 12.32 (1H, d, *J* 13.4, = C-N*H*-), 13.25 (1H, d, *J* 12.7, =C–N*H*–). ^{13}C NMR (d_6-DMSO, δ, ppm): 24.1 (CH$_3$), 31.3 (CH$_3$), 102.4 (C), 112.2 (CH), 116.4 (CH), 117.3 (CH), 123.5 (CH), 125.6 (CH), 128.1 (CH), 129.2 (CH), 132.2 (C), 140.3 (C), 148.2 (CH), 150.2 (C), 154.5 (C), 158.8 (C=O), 169.4 (C = O), 201.7 (C=O). SM [$C_{18}H_{15}N_3O_3$] [321.33]. Elemental analyses Found (Calcd.) C, 66.75 (67.28); H, 4.62 (4.71); N, 13.51 (13.08).

*3-[(3-Acetylanilino)methylidene]-8-methyl-2H-pyrido[1,2-a]-pyrimidine-2,4-dione (**3**)* Light yellow powder. Mp = 158–160°C. Yield: 78%; IR (ν neat)/cm^{-1}) 3211(m), 3060(m), 1700 (s), 1645(m); ^{1}H NMR (d_6-DMSO, δ, ppm): 2.23 (s, CH$_3$-pyrimidine-2,4-dione), 3.24 (3H, s, C*H*$_3$CO), 7.23 (1H, dd, *J* 7.85, 8.12 Hz, acetylanilino C$_5'$-*H*), 7.36 (1H, dd, *J* 8.1, 2.2 Hz, acetylamino C$_6'$-*H*), 7.38 (1H, d, *J* 7.4 Hz, pyrimidine-2,4-dione C$_7$-*H*), 7.53 (1H, s, *J* 7.4, pyrimidine-2,4-dione C$_9$-*H*), 7.58 (1H, dd, *J* = 7.8, 2.2 Hz, acetylanilino C$_4'$-*H*), 7.69 (1H, s, acetylanilino C$_2'$-*H*), 7.80 (1H, d, *J* 7.4 Hz, pyrimidine-2,4-dione C$_6$-*H*), 9.0 (1H, d, *J* 12.9, = C*H*–N-), 9.20 (1H, d, *J* 13.4, = C*H*–N–), 12.90 (1H, d, *J* = 13.3, = C-N*H*-), 13.18 (1H, d, *J* 12.9, = C–N*H*–); ^{13}C NMR (d_6-DMSO, δ, ppm): 23.8 (CH$_3$), 31.7 (CH$_3$), 104.0 (C), 110.4 (CH), 117.4 (CH), 117.5 (CH), 124.0 (CH), 125.1 (CH), 128.4 (CH), 129.0 (CH), 131.3 (C), 140.8 (C), 147.6 (CH), 151.3 (C), 153.5 (C), 158.4 (C = O), 169.8 (C = O), 200.9 (C = O). SM [$C_{18}H_{15}N_3O_3$] [321.33]. Elemental analyses Found (Calcd.): C, 66.71 (67.28); H, 4.85 (4.71); N, 13.45 (13.08).

*3-[(4-Acetylanilino)methylidene]-8-methyl-2H-pyrido[1,2-a]pyrimidine-2,4-dione (**4**)* Light yellow powder. Mp = 208–210°C. Yield: 76%; IR (ν neat)/cm^{-1}) 3209(m), 3060(m), 1703 (s), 1645(m); ^{1}H NMR (d_6-DMSO, δ, ppm): 2.24 (3H, s, C*H*$_3$– pyrimidine-2,4-dione), 3.21 (3H, s, C*H*$_3$CO), 7.32 (2H, d, *J* 8.24 Hz, acetlanilino C$_2'$,$_6'$-*H*), 7.40 (1H, d, *J* 7.43 Hz, pyrimidine-2,4-dione C$_7$-*H*), 7.54 (1H, s, pyrimidine-2,4-dione C$_9$-*H*), 7.62 (2H, d, *J* 8.24 Hz, acetylanilino C $_3'$,$_5'$-*H*), 7.81 (1H, d, *J* 7.4 Hz, pyrimidine-2,4-dione C$_6$-*H*), 8.98 (1H, d, *J* 13.0, =C*H*–N–), 9.22 (1H, d, *J* 13.6, =C*H*–N–), 12.92 (1H, d, *J* 13.2, = C-N*H*-), 13.22 (1H, d, *J* 13.0, =C–N*H*–); ^{13}C NMR (d_6-DMSO, δ, ppm): 24.4 (CH$_3$), 33.0 (CH$_3$), 103.1 (C), 109.6 (CH), 119.0 (CH), 119.2 (CH), 121.3 (CH), 128.9 (CH), 130.5 (C), 139.9 (C), 148.0 (CH), 150.8 (C), 154.0 (C), 154.6 (C=O), 170.4 (C=O), 201.8 (C=O). SM [$C_{18}H_{15}N_3O_3$] [321.33]. Elemental analyses Found (Calcd.) C, 67.65 (67.28); H, 4.35 (4.71); N, 12.92 (13.08).

*3-[(Mesitylamino)methylidene]-8-methyl-2H-pyrido[1,2-a]pyrimidine-2,4-dione (**5**)* White powder. Mp = 238–240°C. Yield 70%; IR (ν neat)/cm^{-1}) 3279(m), 3080(m), 1700 (s), 1685(m); ^{1}H NMR (d_6-DMSO, δ, ppm): 2.16 (3H, s, *p*-C*H*$_3$), 2.18 (6H, s, 2 × *o*-CH$_3$), 2.20 (3H, s, C*H*$_3$-pyrimidine-2,4-dione), 7.00 (2H, s, mesitylamino C$_3'$,$_5'$-*H*), 7.35 (1H, d, *J* 7.5 Hz, pyrimidine-2,4-dione C$_7$-*H*), 7.44 (1H, s, pyrimidine-2,4-dione C$_9$-*H*), 7.72 (1H, d, *J* 7.5 Hz, pyrimidine-2,4-dione C$_6$-*H*), 8.88 (1H, d, *J* 13.5, =C*H*–N–), 9.15 (1H, d, *J* 13.3, =C*H*–N–), 11.32 (1H, d, *J* 13.1, = C–N*H*–), 12.45 (1H, d, *J* 13.5, =C–N*H*–);^{13}C NMR (d_6-DMSO, δ, ppm): 18.5(CH$_3$), 20.8(2 × CH$_3$), 23.3 (CH$_3$), 103.9 (C), 111.6 (CH), 115.0 (CH), 130.2

(CH), 131.3 (CH), 132.7 (C), 136.9 (C), 138.9 (CH), 150.5 (C), 151.9 (C), 160.8 (C=O), 166.7 (C=O). SM [$C_{19}H_{19}N_3O_2$] [321.37]. Elemental analyses Found (Calcd.) C, 69.50 (71.01); H, 6.22 (5.96); N, 12.72 (13.08).

*3-[(2-Fluoroanilino)methylidene]-8-methyl-2H-pyrido[1,2-a]-pyrimidine-2,4-dione (**6**)* Maroon powder. Mp = 114–116°C. Yield 68%; IR (ν neat)/cm^{-1}) 3211(m), 3029(m), 1709 (s), 1675(m); ^{1}H NMR (d_6-DMSO, δ, ppm): 2.21 (3H, s, CH_3-pyrimidine-2,4-dione), 7.10 (1H, ddd, *J* 7.7, 7.4, 2.1 Hz fluoroanilino C_4'–*H*), 7.18 (1H, dd, *J* 7.8, 2.15, fluoroanilino C_6'–*H*),7.24 (1H, ddd, *J* 7.8, 7.4, 2.1 fluoroanilino C_5'–*H*), 7.30 (dd, 1H, *J* 7.7, 2.1 Hz, fluoroanilino C_3'–H), 7.37 (s, *J* 7.37, pyrimidine-2,4-dione C_7–H), 7.52 (1H, s, pyrimidine-2,4-dione C_9–*H*), 7.79 (2H, d, *J* 7.3, pyrimidine-2,4-dione $C_{6,7}$–*H*), 8.92 (1H, d, *J* 13.4, = C*H*–N–), 9.20 (1H, d, *J* 13.8, = C*H*–N–), 11.45 (1H, d, *J* 13.6, = C–N*H*–), 12.88 (1H, d, *J* 13.4, = C–N*H*–); ^{13}C NMR (d_6-DMSO, δ, ppm): 23.2(CH_3), 105.6(C), 111.0 (CH), 115.1 (CH), 115.7 (CH), 115.8 (CH), 124.2 (CH), 124.3 (CH), 125.9 (C), 132.03 (CH), 142.5 (CH), 153.8 (C), 154.7(C), 156.7(C)161.4 (C = O), 166.4 (C = O). SM [$C_{16}H_{12}FN_3O_2$] [297.28]. Elemental analyses Found (Calcd.) C, 65.01 (64.64); H, 3.83 (4.07); N, 13.65 (14.13).

*3-[(3-Fluoroanilino)methylidene]-8-methyl-2H-pyrido[1,2-a]-pyrimidine-2,4-dione (**7**)* Yellowish Brown powder. Mp = 174–176°C. Yield 69%; IR (ν neat)/cm^{-1}) 3259(m), 3064(m), 1710 (s), 1645(m); ^{1}H NMR (d_6-DMSO, δ, ppm): 2.20 (3H, s, CH_3-pyrimidine-2,4-dione), 7.12 (1H, dd, *J* 7.65, 2.20 Hz, fluoroanilino C_6'–*H*), 7.19 (1H, dd, *J* 7.6, 2.20 H_Z, fluoroanilino C_4'–*H*), 7.27 (1H, s, fluoroanilino C_2'–*H*), 7.34 (1H, dd, *J* 7.6, 7.6 H_Z, fluoroanilino C_5'–*H*), 7.36 (1H, d, *J* 7.39, pyrimidine-2,4-dione C_7–*H*), 7.51 (1H, s, pyrimidine-2,4-dione C_9–*H*), 7.78 (1H, d, *J* 7.3, pyrimidine-2,4-dione C_6–*H*), 8.83 (1H, d, *J* 13.5, = C*H*–N–), 9.10 (1H, d, *J* 13.4, = C*H*–N–), 11.50 (1H, d, *J* 13.3, = C–N*H*–), 12.80 (1H, d, *J* 13.5, = C–N*H*–); ^{13}C NMR (d_6-DMSO, δ, ppm): 22.11(CH_3), 106.2(C), 110.0(CH), 110.7 (CH), 112.3 (CH), 115.8 (CH), 127.3 (CH), 127.9 (CH), 132.1 (CH), 138.8 (C), 148.3 (CH), 153.8 (C), 156.3(C), 156.7(C), 160.9 (C = O), 165.8 (C = O). SM [$C_{16}H_{12}FN_3O_2$] [297.28]. Elemental analyses Found (Calcd.) C, 64.23 (64.64); H, 4.44 (4.07); N, 13.75 (14.13).

*3-[(4-Fluoroanilino)methylidene-8-methyl-2H-pyrido[1,2-a] pyrimidine-2,4-dione (**8**)* Shiny dirty green powder. Mp = 132–134°C. Yield 81%; IR (ν neat)/cm^{-1}) 3260(m), 3042(m), 1725 (s), 1640(m); ^{1}H NMR (d_6-DMSO, δ, ppm): 2.18 (3H, s, CH_3-pyrimidine-2,4-dione), 7.11 (2H, d, *J* 7.2 H_Z, fluoroanilino C $_{2',6'}$–*H*), 7.25 (2H, d, *J* 7.23 H_Z, fluoroanilino C $_{3',5'}$–*H*), 7.33 (1H, d, *J* 7.4, pyrimidine-2,4-dione C_7–*H*), 7.49 (1H, s, pyrimidine-2,4-dione C_9–*H*), 7.76 (1H, d, *J* 7.4, pyrimidine-2,4-dione C_6–*H*), 8.75 (1H, d, *J* 13.2, = C*H*–N–), 9.0 (1H, d, *J* 13.5, = C*H*–N–), 11.44 (1H, d, *J* 13.0, = C–N*H*–), 12.76 (1H, d, *J* 13.2, = C–N*H*–); ^{13}C NMR (d_6-DMSO, δ, ppm): 22.11(CH_3), 104.7(C), 110.3(CH), 115.8 (CH), 116.1 (CH), 117.9 (CH), 132.0 (CH), 136.3 (C), 148.2 (CH), 153.8 (C), 156.3(C), 156.9(C), 160.6 (C = O), 166.2 (C = O). SM [$C_{16}H_{12}FN_3O_2$] [297.28]. Elemental analyses Found (Calcd.) C, 64.92 (64.64); H, 3.77 (4.07); N, 13.69 (14.13).

*8-Methyl-3-[(2-sulfanylanilino)methylidene]-2H-pyrido[1, 2-a]pyrimidine-2,4-dione (**9**)* Light green powder. Mp = 218–220°C. Yield 75%; IR (ν neat)/cm^{-1}) 3270(m), 3007(m), 1716 (s), 1660(m); ^{1}H NMR (d_6-DMSO, δ, ppm): 2.18 (3H, s, CH_3-pyrimidine-2,4-dione), 4.22 (1H, s, –S*H*), 7.10–7.22(3H, m, sulfanylanilino $C_{3',4',6'}$–*H*), 7.32 (1H, d, *J* 7.5, pyridopyrimidine-2,4-dione C_7–*H*), 7.42 (1H, s, pyridopyrimidine-2,4-dione C_9–*H*), 7.70 (1H, d, *J* 7.5, pyridopyrimidine-2,4-dione C_6–*H*), 8.62 (1H, d, *J* 13.8, = C*H*–N–), 9.90 (1H, d, *J* 13.5, = C*H*–N–), 11.22 (1H, d, *J* 13.2, = C–N*H*–), 12.35 (1H, d, *J* 13.8, = C–NH–) ^{13}C NMR (d_6-DMSO, δ, ppm): 21.5(CH_3), 105.8(C), 110.0(CH), 114.3 (CH), 115.8 (CH), 118.3 (C), 118.4 (CH), 127.0 (CH), 131.9 (CH), 133.0 (CH), 147.3 (C), 151.8 (C), 155.3(CH), 156.9(C), 162.5 (C = O), 167.2 (C = O). SM [$C_{16}H_{13}N_3O_2S$] [311.36]. Elemental analyses Found (Calcd.) C, 62.10 (61.72); H, 3.88 (4.21); N, 13.21 (13.50).

*8-Methyl-3-{[(4-methyl-2-pyridinyl)amino]methylidene}-2H-pyrido[1,2-]pyrimidine-2,4-dione (**10**)* Shiny brown crystals. Mp = 168–170°C. Yield 66%; IR (ν neat)/cm^{-1}) 3288(m), 3010(m), 1706 (s), 1680(m); ^{1}H NMR (d_6-DMSO, δ, ppm): 2.26 (3H, s, CH_3-pyrimidine-2,4-dione), 2.30 (3H, s, pyridinyl CH_3), 7.42 (1H, d, *J* 7.48 H_Z, pyrimidine-2,4-dione C_7–*H*), 7.48 (1H, d, *J* 7.5, pyridinyl C_5'–*H*), 7.57 (1H, s, pyrimidine-2,4-dione C_9–*H*), 7.84 (1H, d, *J* 7.4 H_Z, pyrimidine-2,4-dione C_6–*H*), 7.88 (1H, s, pyridinyl C_3'–*H*), 8.20 (1H, d, *J* 7.5, pyridinyl C_6'–*H*), 8.72 (1H, d, *J* 12.8, = C*H*–N–), 9.32 (1H, d, *J* 13.1, = C*H*–N–), 12.21 (1H, d, *J* 12.9, = C–N*H*–), 13.42 (1H, d, *J* 12.8, = C–N*H*–). ^{13}C NMR (d_6-DMSO, δ, ppm): 21.8 (CH_3), 23.0 (CH_3), 107.7 (C), 115.3 (CH), 119.7 (CH), 121.8 (CH), 123.4 (CH), 124.0 (CH), 134.3 (CH), 143.9 (CH), 147.1 (C), 148.3 (C), 152.8(C), 155.4 (C), 164.2 (C = O), 167.1 (C = O). SM [$C_{16}H_{14}N_4O_2$] [294.31]. Elemental analyses Found (Calcd.) C, 64.74 (65.3); H, 5.31 (4.79); N, 19.55 (19.04).

*8-Methyl-3-{[(5-methyl-2-pyridinyl)amino] methylidene}-2H-pyrido[1,2-]pyrimidine-2,4-dione (**11**)* Shiny light yellow. Mp = 179–181°C. Yield 68%; IR (ν neat)/cm^{-1})

3269(m), 3010(m), 1730 (s), 1685(m); ^{1}H NMR (d_6-DMSO, δ, ppm): 2.26 (3H, s, CH_3-pyrimidine-2,4-dione), 2.32 (3H, s, pyridinyl CH_3), 7.41 (1H, d, J 7.4 Hz, pyrimidine-2,4-dione C_7–H), 7.56 (1H, s, pyrimidine-2,4-dione C_9–H), 7.76 (1H, d, J 7.5 Hz, pyridinyl C_3'–H), 7.84 (1H, d, J 7.4 Hz, pyrimidine-2,4-dione C_6–H), 7.96 (1H, d, J 7.5 Hz, pyridinyl C_4'–H), 8.10 (1H, s, pyridinyl C_6'–H), 8.82 (1H, d, J 12.3, = CH–N–), 9.30 (1H, d, J 12.9, = CH–N–), 12.19 (1H, d, J 12.7, = C–NH–), 13.41 (1H, d, J 12.3, = C–NH–); ^{13}C NMR (d_6-DMSO, δ, ppm): 18.4 (CH_3), 21.7 (CH_3), 105.2 (C), 111.0(CH), 112.3 (CH), 114.6 (CH), 123.5 (C), 130.4 (CH), 135.6 (CH), 143.8 (CH), 152.7 (CH), 152.8 (C), 153.0 (C), 154.5(C), 164.2 (C = O), 167.1 (C = O). SM [$C_{16}H_{14}N_4O_2$] [294.31]. Elemental analyses Found (Calcd.) C, 65.70 (65.30); H, 5.11 (4.79); N, 18.87 (19.04).

*8-Methyl-3-{[(5-nitro-2-pyridinyl)amino] methylidene}-2H-pyrido[1,2-a]pyrimidine-2,4-dione (**12**)* Golden Yellow powder. Mp = 156–158°C. Yield 72%; IR (ν neat)/cm^{-1}) 3267(m), 3034(m), 1706 (s), 1665(m); ^{1}H NMR (d_6-DMSO, δ, ppm): 2.27 (3H, s, C$\boldsymbol{H_3}$-pyrimidine-2, 4-dione), 7.43 (1H, d, J 7.4 Hz, pyrimidine-2,4-dione C_7–H), 7.59 (1H, s, pyrimidine-2,4-dione C_9–H), 7.85 (1H, d, J 7.4 Hz, pyrimidine-2,4-dione C_6–H), 8.20 (1H, d, J 7.4 Hz, pyridinyl C_3'–H), 8.88 (1H, d, J 7.45 Hz, pyridinyl C_4'–H), 9.21 (1H, s, pyridinyl C_6'–H), 8.91 (1H, d, J 12.3, = CH–N–), 9.45 (1H, d, J 13.2, = CH–N–), 12.72 (1H, d, J 12.9, = C–NH–), 13.68 (1H, d, J 12.3, = C–NH–); ^{13}C NMR (d_6-DMSO, δ, ppm): 18.6 (CH_3), 21.0 (CH_3), 104.6 (C), 110.2 (CH), 110.8 (CH), 115.8(CH), 132.1 (CH), 134.9(CH), 139.1 (C), 146.8 (CH), 147.7 (CH), 153.8 (C), 156.7(C), 158.9(C), 164.2 (C = O), 167.1 (C = O). SM [$C_{15}H_{11}N_5O_4$] [325.28]. Elemental analyses Found (Calcd.) C, 55.02 (55.39); H, 3.66 (3.41); N, 20.16 (21.53).

*N'-{[8-Methyl-2,4-dioxo-2H-pyrido[1,2-a]pyrimidin-3(4H)-ylidene]methyl}-benzo-hydrazide (**13**)* White crystals. Mp = 182–184°C. Yield 78%; IR (ν neat)/cm^{-1}) 3275(m), 3021(m), 1718 (s), 1651(m); ^{1}H NMR (d_6-DMSO, δ, ppm): 2.23 (3H, s, CH_3-pyrimidine-2,4-dione), 7.33 (2H, dd, J 7.35, 7.42 Hz, benzohydrazide C_3',$_5'$-H), 7.38 (1H, d, J 7.67 Hz, pyrimidine-2,4-dione C_7–H),7.48 (1H, s, pyrimidine-2,4-dione C_9–H),7.51 (1H, t, J 7.4, 2.0 Hz, benzohydrazide C_4'–H),7.78 (1H, d, J 7.6 Hz, pyrimidine-2,4-dione C_6–H), 7.88 (2H, dd, J 7.35, 2.0 Hz, benzohydrazideC_2',$_6'$–H), 8.45 (1H, d, J 13.4, = CH–N–), 8.75 (1H, d, J 13.2, = CH–N–), 11.35 (1H, d, J 13.8, = C–NH–N–C = O), 11.96 (1H, s, –N–NH–C = O), 12.45 (1H, d, J 13.4, = C–NH–N–C = O). ^{13}C NMR (d_6-DMSO, δ, ppm): 20.5 (CH_3), 107.3 (C), 112.4 (CH), 113.7 (CH), 122.4 (CH), 122.8 (CH), 124.2 (CH), 126.7 (CH), 128.0 (CH), 136.3 (C), 143.8 (C), 153.5 (C = O), 160.7 (C = O), 174.2 (C = O). SM [$C_{17}H_{14}N_4O_3$] [322.32]. Elemental analyses Found (Calcd.) C, 62.89 (63.35); H, 4.75 (4.38); N, 17.11 (17.38).

*4-Nitro-N′-{[8-methyl-2,4-dioxo-2H-pyrido[1,2-a]pyrimidin-3(4H)-ylidene]methyl} benzohydrazide (**14**)* Orange brown powder. Mp = 196–198°C. Yield 74%; IR (ν neat)/cm^{-1}) 3235(m), 3010(m), 1720 (s), 1685(m); ^{1}H NMR (DMSO-d_6, δ, ppm): 2.25 (3H, s, CH_3-pyrimidine-2,4-dione),7.41 (1H, d, J 7.70, pyrimidine-2,4-dione C_7–H), 7.52 (1H, s, pyrimidine-2,4-dione C_9–H), 7.82 (1H, d, J 7.70 Hz, pyrimidine-2,4-dione C_6–H), 8.21 (2H, d, J 7.5 Hz, benzohydrazide C_2',$_6'$–H), 8.32 (2H, d, J 7.5 Hz, benzohydrazide C_3',$_5'$–H), 8.82 (1H, d, J 12.2, = CH–N–), 9.45 (1H, d, J 12.8, = CH–N–), 12.0 (1H, s, –N–NH–C = O), 12.55 (1H, d, J 12.5, = C–NH–N–C = O), 13.40 (1H, d, J 12.2, = C–NH–N–C = O); ^{13}C NMR (d_6-DMSO, δ, ppm): 20.1 (CH_3), 107.5 (C), 109.8 (CH), 114.6 (CH), 124.7 (CH), 127.0 (CH), 133.6 (CH), 139.6 (C), 146.0(CH), 151.0 (C), 152.6 (C), 156.0 (C), 159.8 (C = O), 161.3 (C = O), 173.1 (C = O). SM [$C_{17}H_{13}N_5O_5$] [367.32]. Elemental analyses Found (Calcd.) C, 55.22 (55.59); H, 3.98 (3.57); N, 20.33 (19.07).

*4-Bromo-N′-{[8-methyl-2,4-dioxo-2H-pyrido[1,2-a]pyrimidin-3(4H)-ylidene]methyl}benzohydrazide (**15**)* White crystals. Mp = 212–214°C. Yield 70%; IR (ν neat)/cm^{-1}) 3290(m), 3010(m), 1735 (s), 1670(m); ^{1}H NMR (d_6-DMSO, δ, ppm): 2.22 (3H, s, CH_3-pyrimidine-2,4-dione), 7.34 (1H, d, J 7.72 Hz, pyrimidine-2,4-dione C_7–H), 7.43 (2H, d, J 7.42 Hz, benzohydrazide C_3',$_5'$–H), 7.45 (1H, s, pyrimidine-2,4-dione C_9–H), 7.74 (1H, d, J 7.72 Hz, pyrimidine-2,4-dione C_6–H), 7.90 (2H, d, J 7.4 Hz, benzohydrazide C_2',$_6'$–H), 8.56 (1H, d, J 13.7, = CH–N–), 8.85 (1H, d, J 14.2, = CH–N–), 11.40 (1H, d, J 13.9, = C–NH–N–C = O), 11.93 (1H, s, –N–NH–C = O), 12.65 (1H, d, J 13.7, = C–NH–N–C = O); ^{13}C NMR (d_6-DMSO, δ, ppm): 20.1 (CH_3), 107.0 (C), 112.0 (CH), 116.0 (CH), 127.2 (C), 129.5 (CH), 129.8(C), 130.6(CH), 132.2 (CH), 148.0(CH), 154.1 (C), 156.9 (C), 158.7 (C = O), 162.6 (C = O), 170.0 (C = O). SM [$C_{17}H_{13}BrN_3O_3$] [401.21]. Elemental analyses Found (Calcd.) C, 50.39 (50.89); H, 3.40 (3.27); N, 13.51 (13.96).

*4-Chloro-N′-{[8-methyl-2,4-dioxo-2H-pyrido[1,2-a]pyrimidin-3(4H)-ylidene]methyl}benzohydrazide (**16**)* White powder. Mp = 198–200°C. Yield 98% as white solid; IR (ν neat)/cm^{-1}) 3210(m), 3011(m), 1718 (s), 1666(m); ^{1}H NMR (d_6-DMSO, δ, ppm): 2.23 (3H, s, CH_3-pyrimidine-2,4-dione), 7.37 (1H, d, J 7.68 Hz pyrimidine-2,4-dione C_7–H), 7.45 (2H, d, J 7.4 Hz, benzohydrazide C_3',$_5'$–H), 7.48 (1H, s, pyrimidine-2,4-dione C_9–H), 7.76 (1H, d, J 7.6 Hz, pyrimidine-2,4-dione C_6–H), 7.92 (2H, d,

J 7.4 Hz, benzohydrazide $C_{2',6'}$–*H*), 8.60 (1H, d, *J* 13.6, = C*H*–N–), 8.90 (1H, d, *J* 13.9, = C*H*–N–), 11.50 (1H, d, *J* 13.7, = C–N*H*–N–C = O), 11.94 (1H, s, –N–N*H*–C = O), 12.67 (1H, d, *J* 13.6, = C–N*H*–N–C = O); ^{13}C NMR (d_6-DMSO, δ, ppm): 23.0 (CH_3), 106.6 (C), 112.3 (CH), 116.0 (CH), 127.9(CH), 128.2 (CH), 129.0(CH), 132.1(C), 142.2(C), 145.6(CH), 150.7 (C), 154.4 (C), 159.6 (C = O), 163.5 (C = O), 172.6 (C = O). SM [$C_{17}H_{13}ClN_4O_3$] [356.76]. Elemental analyses Found (Calcd.) C, 56.88 (57.23); H, 3.79 (3.67); N, 15.23 (15.70).

*4-Hydroxy-N′-{[8-methyl-2,4-dioxo-2H-pyrido[1,2-a]pyrimidin-3(4H)-ylidene]methyl}benzohydrazide (**17**)* White crystals. Mp = 210–212°C. Yield 78%; IR (*v* neat)/cm^{-1}) 3275(m), 3013(m), 1710 (s), 1665(m); ^{1}HNMR (d_6-DMSO, δ, ppm): 2.21 (3H, s, C*H*$_3$-pyrimidine-2,4-dione), 7.32 (1H, d, *J* 7.73 Hz pyrimidine-2,4-dione C_7-*H*), 7.39 (2H, d, *J* 7.5 Hz, benzohydrazide $C_{3',5'}$–*H*), 7.44 (1H, s, pyrimidine-2,4-dione C_9–*H*), 7.73 (1H, d, *J* 7.73 Hz, pyrimidine-2,4-dione C_6–*H*), 7.94 (2H, d, *J* 7.5 Hz, benzohydrazide $C_{2',6'}$–*H*), 9.75 (1H, s, benzohydrazide –O*H*), 8.44 (1H, d, *J* 13.8, = C*H*–N–), 8.83 (1H, d, *J* 14.2, = C*H*–N–), 11.42 (1H, d, *J* 14.0, = C–N*H*–N–C = O), 11.88 (1H, s, –N–N*H*–C = O), 12.60 (1H, d, *J* 13.8, = C–N*H*–N–C = O); ^{13}C NMR (d_6-DMSO, δ, ppm): 23.1 (CH_3), 110.0 (C), 110.3 (CH), 115.7 (CH), 115.8(CH), 129.4(C), 128.1 (CH), 128.7(CH), 142.4(CH), 154.3 (C), 156.0 (C), 162.3 (C = O), 163.1(C), 165.7 (C = O), 170.2 (C = O). SM [$C_{17}H_{14}N_4O_4$] [338.32]. Elemental analyses Found (Calcd.) C, 59.88 (60.35); H, 4.35 (4.17); N, 16.19 (16.56).

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