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Original article

Synthesis and biological evaluation of some novel pyrido[1,2-*a*] pyrimidin-4-ones as antimalarial agents

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ABSTRACT

Novel pyrido[1,2-*a*]pyrimidin-4-ones have been synthesized and evaluated for their antimalarial activity by SYBR Green I assay against erythrocytic stages of chloroquine (CQ) sensitive *Pf* 3D7 strain. The antimalarial screening of 42 different compounds revealed that 3-Fluorobenzyl(4-oxo-4*H*-pyrido [1,2-*a*] pyrimidin-3-yl)carbamate (**21**, IC_{50} value 33 μM) and 4-Oxo-*N*-[4-(trifluoromethyl)benzyl]-4*H*-pyrido [1,2-*a*]pyrimidine-3-carboxamide (**37**, IC_{50} value 37 μM) showed moderate antimalarial activity. Cytotoxicity study was performed against mammalian cell line (Huh-7) by using the MTT assay for the moderately active compounds. Structural activity relationship (SAR) studies displayed that B-ring unsubstituted pyrido[1,2-*a*]pyrimidine scaffold is responsible for the antimalarial activities of the evaluated derivatives. This SAR based antimalarial screening supported that pyrido[1,2-*a*]pyrimidin-4-one can be considered as a lead heterocyclic structure for further development of more potent derivatives for antimalarial activity.

1. Introduction

Malaria, a tropical disease caused by protozoan parasites of the genus *Plasmodium*, has been of great concern to tropical countries and is now a cause of concern to more than 40% of the world's population [1]. As per the recent WHO report malaria is having devastating impact on the lives of more than 3 billion people of the world, about half of the humanity [2]. There is an urgent and compelling need for the discovery of new antimalarial drugs to save lives and reduce morbidity and mortality [3]. The existing antimalarial drugs are not adequate to combat malaria, primarily because of resistance developed by the parasite. The drug resistance associated parasitic mutations to antimalarial drugs in the malaria parasite is a major contributor to the re-emergence of the disease and its spread in new locations and populations. The problem has got further compounded due to non-enrichment of antimalarial drug inventory [4]. Unfortunately an effective antimalarial vaccine is also not available. WHO aims for the global roll back of malaria with intensified efforts in providing access to affordable, safe and effective antimalarial treatments worldwide along with preventive measures [2].

A large number of newer motifs like chalcones [5,6], (thio)semicarbazones [7] and phenothiazines [8] have been reported in the literature to exhibit antimalarial activity. Unfortunately compounds from none of these classes have crossed the clinical phase of evaluation as antimalarial agents. There are reports of some heterocyclic [9] and polycyclic pyrimidines [10] exhibiting antimalarial activity. Recently some of the potent antimalarial compounds containing 4-Oxymethyl-1,2-dioxanes [14], 4-amino-quinoline-pyrimidine [12] and quinolines trizoles [13] have been reported. Taking the lead information from literature we have synthesized and reported [11] some compounds of pyrido[1,2-*a*]pyrimidin-4-one class of heterocycles (Fig. 1). In continuation of our previous work more novel compounds belonging to the four series-(**I** to **IV**) of pyrido[1,2-*a*] pyrimidin-4-ones were synthesized (Fig. 1) and evaluated against *Plasmodium falciparum* for their antimalarial potential.

2. Results and discussion

2.1. Chemistry

The targeted compounds were prepared by coupling of suitable isocyanate [14] (**1**) with amine derivatives of type R'NHA in toluene under reflux conditions following the procedure as depicted in

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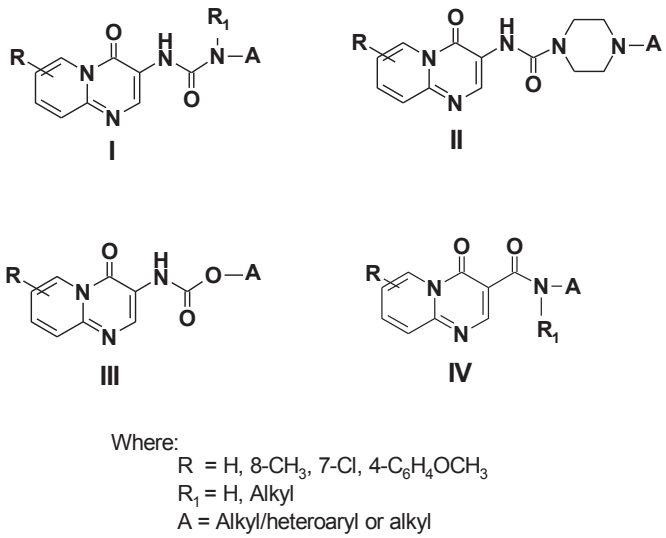


Fig. 1. Pyrido[1,2-*a*]pyrimidin-4-one (**Series-I** to **IV**) derivatives synthesized for potential antimalarial activity.

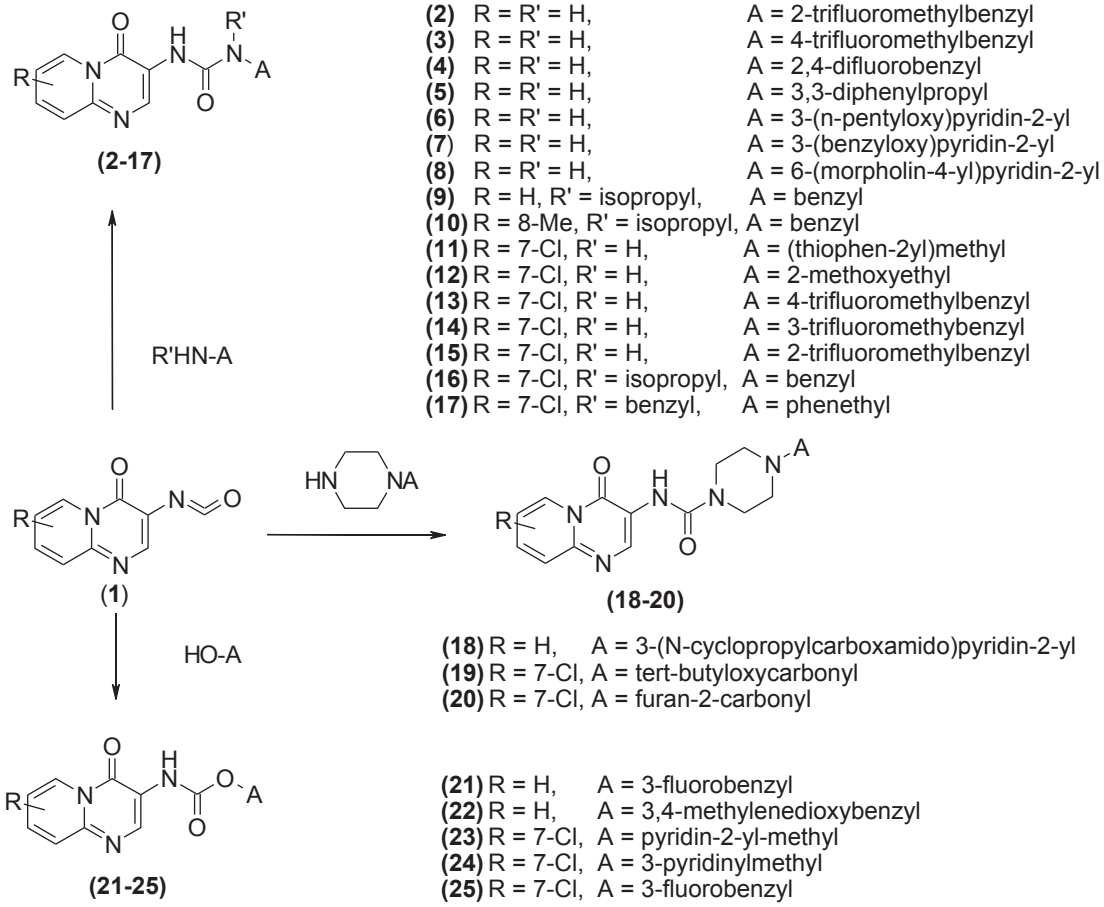
Scheme 1 to get the ureas (**2**–**17**). *N*-(4-Oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)-4-(pyridin-2-yl)piperrazine-1-carboxamide derivatives (piperazine urea analogs) of the series-II (**18**–**20**) were synthesized by coupling of the isocyanate with suitably substituted piperazine derivatives under reflux conditions in toluene as depicted in Scheme 1. The carbamates (**21**–**25**) were synthesized by coupling of suitable isocyanate (**1**) with aryl alcohols in toluene under reflux conditions [14] as depicted in Scheme 1. Some compounds bearing ester (**26**, **27**), amide (**28**, **29**) and nitrile (**30**, **31**) groups were also synthesized following the reaction sequence as outlined in Scheme 2.

The amides (**33**–**49**) were prepared by EDC coupling of a suitable acid derivative [14] (**32**) with suitable amine derivatives following Scheme 3. The isocyanate derivatives (**1**) were synthesized following the synthetic schemes reported in our earlier publication [14].

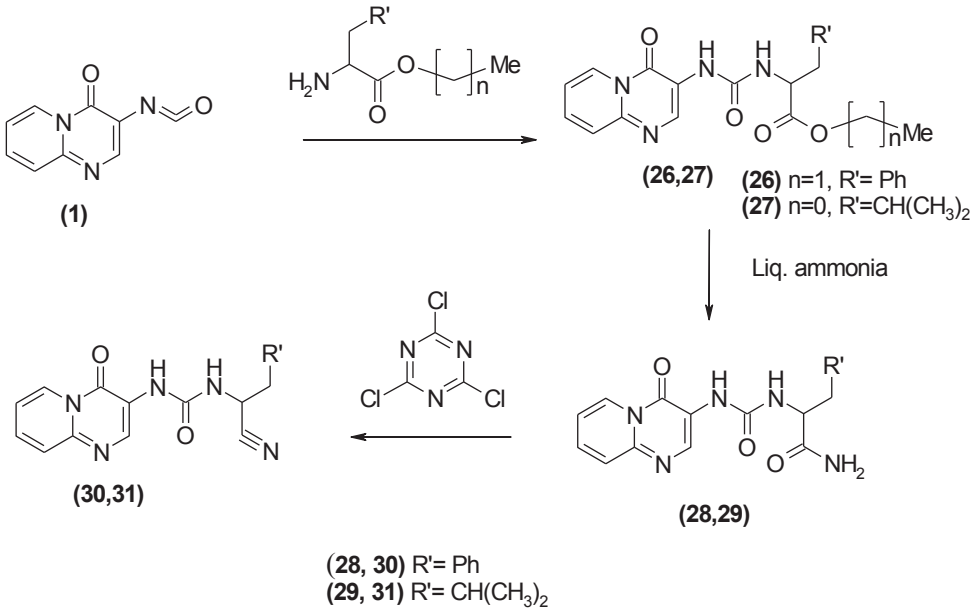
In order to study the hydrophobic binding interactions it was planned to prepare some compounds having aryl ring attached to the B-ring (i.e. pyridine) of the pyridopyrimidine ring. Such compounds (**53**–**56**) were synthesized by following the reaction sequence outlined in Scheme 4. The ester (**50**) on Suzuki coupling with *p*-methoxyphenylboronic acid yielded the ester (**51**) which on acid hydrolysis gave the acid (**52**). The acid (**52**) on EDC coupling with different amines offered compounds of type (**IV**) i.e. **53**–**56**.

2.2. Biological evaluation

The antimalarial activity of compounds (series **I**–**IV**) was measured by SYBR Green I Assay against *P. falciparum* (3D7) and the 50% inhibitory concentrations (IC_{50}) are given in Table 1. Different substituents on the basic moiety of pyrido[1,2-*a*]pyrimidin-4-ones have been explored to ascertain the structure activity relationship (SAR) among the synthesized compounds. Analysis of the results (Tables 1and 2) revealed some interesting points.



Scheme 1. Synthesis of ureas (**2**–**17**), piperazine urea analogs (**18**–**20**) and carbamate derivatives (**21**–**25**).

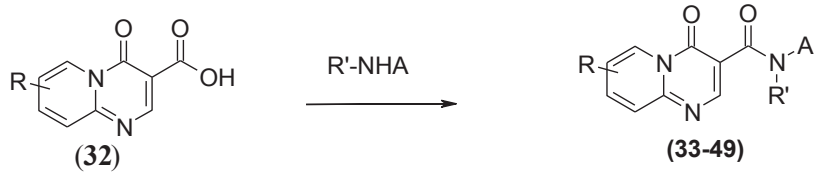


Scheme 2. Synthesis of ester (**26**–**27**), amide (**28**, **29**) and nitrile (**30**, **31**) derivatives.

Broadly two types of substitutions have been performed in the basic pyrido[1,2-*a*]pyrimidin-4-one ring system i.e. substitution in the pyridine ring either at position 7 or 8 and substitution at position 3 in the pyrimidine ring of the pyrido[1,2-*a*]pyrimidinone ring skeleton. Three types of functional groups have been attached at C-3 position in the pyrimidine ring i.e. 1,3-disubstituted or 1,3,3-trisubstituted urea, N-substituted carboxamide and carbamate. Considering an IC_{50} of >100 μM in the category of inactives it can be assumed that majority of the compounds are poorly active or inactive. But some interesting observations could be made from the study of Table 1. Substitution of 7-chloro or 8-methyl/8-(4-methoxyphenyl) groups made the whole moiety inactive as some of the compounds (**9**, **21**, **34**, **36**, **37** and **38**) having no substituents at C-7 or C-8 showed antimalarial activity although of moderate potency. Amongst the three functional groups attached at C-3 position, the carboxamide offered the highest number (**34**, **36**, **37**, **59**, **70**) of such compounds. One of the carbamates yielded the most potent compound (**21**) amongst the whole lot inspite of the fact that lesser number of carbamates were synthesized and evaluated in comparison to the urea and carboxamide derivatives. Biological inertness of urea derivatives was a bit surprise and disappointment as this group had offered active compounds as falcipain-2/3 inhibitors as reported in our earlier publication [14]. Substitution of 8-(4-methoxyphenyl) on the pyrido[1,2-*a*]pyrimidine ring proved to be another set back as earlier we had observed that 7-Cl and 8-Me substituted derivatives yielded active falcipain-2/3 inhibitors among the series of urea derivatives. Another interesting feature of the study is that all the active compounds possessed fluoro/trifluoromethyl groups attached to the benzyl moiety.

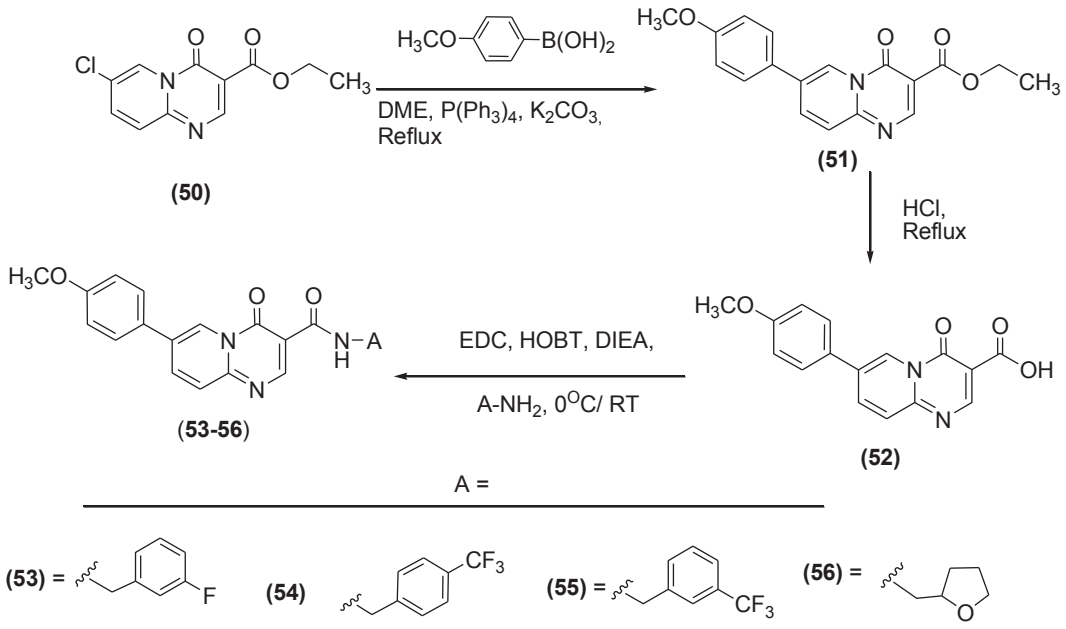
In nutshell, the bare pyrido[1,2-*a*]pyrimidin-4-one ring moiety substituted with a carbamate function at C-3 position having 3-fluorobenzyl side chain offered the most active compound (**21**) The B-ring (i.e. pyridine) unsubstituted moiety is preferred over a substituted one for antimalarial activity and carbamate or carboxamido groupings offered the most active compounds.

Compounds (**21**, **36**, **38** and **47**) showing antimalarial activity in the range of 33–70 μM were further evaluated for their cytotoxicity potential by MTT assay against mammalian cell lines (Huh-7). A selectivity index was calculated by dividing the IC_{50} value obtained in mammalian cell by the IC_{50} value obtained in *pf* 3D7.



(33) R = R' = H, A = 4-methoxybenzyl
(34) R = R' = H, A = 3-fluorobenzyl
(35) R = R' = H, A = 2-trifluoromethylbenzyl
(36) R = R' = H, A = 3-trifluoromethylbenzyl
(37) R = R' = H, A = 4-trifluoromethylbenzyl
(38) R = R' = H, A = 2,4-difluorobenzyl
(39) R = R' = H, A = 2-(morpholin-4-yl)ethyl
(40) R = R' = H, A = 3-(n.pentyloxy)pyridin-2-yl
(41) R = R' = H, A = 3-(benzyloxypyridin-2-yl
(42) R = R' = H, A = (2,5-diethoxy)-4-morpholin-4-ylphenyl
(43) R = R' = H, A = 2,5-dimethyl-1,2-oxazol-4-yl
(44) R = R' = H, A = 1-(4-methoxyphenyl)ethyl
(45) R = R' = H, A = 1-benzylpiperidin-4-yl
(46) R = H, R' = cyclopropyl, A = 2-fluorobenzyl
(47) R = 8-Me, R' = H, A = 4-trifluoromethylbenzyl
(48) R = 7-Cl, R' = H, A = 4-trifluoromethylbenzyl
(49) R = 7-Cl, R' = cyclopropyl, A = 2-fluorobenzyl

Scheme 3. Synthesis of amide derivatives (**33**–**49**).



Scheme 4. Synthesis of 7-(4-methoxyphenyl) substituted derivatives (**51**–**56**).

Interestingly as shown in Table 2, all these compounds showed selectivity index in the range of 1.4–5.4. Compound (**37**) showed the highest selectivity index of >5.4 amongst the five tested compounds. Compound (**38**, **21**) showed selectivity index of >3 and >3.3 respectively. Compounds (**21** and **36**) also showed selectivity index of >3, whereas compound (**47**) showed >1.4 selectivity towards *Pf* 3D7.

3. Conclusion

In continuation of our efforts to design and synthesize novel antimalarial compounds, we have examined the antiplasmodial activity of forty two pyrido[1,2-*a*]pyrimidin-4-one derivatives by SYBR Green Assay against *Pf* 3D7. Only two compounds viz. 3-fluorobenzyl (4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)carbamate (**21**, IC_{50} value of 33 μM) and 4-oxo-*N*-[4-(trifluoromethyl)benzyl]-4*H*-pyrido[1,2-*a*]pyrimidine-3-carboxamide (**37**, IC_{50} value of 37 μM) showed moderate antimalarial activity in the whole series. Based on the activity profile of the reported compounds it could be concluded that pyrido[1,2-*a*]pyrimidin-4-one ring skeleton can be considered as a lead structure for further chemical optimization for obtaining potential antimalarial compounds.

4. Experimental work

4.1. Chemistry

All the reagents and solvents required for synthesis were purified by general laboratory techniques before use. Compounds were purified by passing them through silica gel H (100–200 mesh) purifying column using mixture of ethyl acetate and hexane or methanol as eluent. Melting points were determined using a Labindia make melting point apparatus (heating block type) and are uncorrected. Purity of the compounds and completion of reactions were monitored by thin layer chromatography (TLC) on silica gel plates (60 F_{254}; Merck), visualizing with ultraviolet light or iodine vapors. The yields reported here are un-optimized. The IR spectra were recorded using KBr disc method on a Bruker FT-IR, model alpha. The ^1H and ^{13}C NMR spectra (on a Bruker 400 MHz spectrometer) were recorded in DMSO-d_6/$CDCl_3$ (chemical shifts in δ ppm). The assignments of exchangeable protons were confirmed by the D_2O exchange studies wherever required. Mass spectral data was obtained on a Waters Micromass ESCi spectrometer. Elemental analyses were performed on Thermo fisher scientific (Flash 2000) analyzer. Synthesis of isocyanate derivatives (**1**), acids (**32**) and ethyl 7-chloro-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidine-3-carboxylate (**50**) was carried out as per the reported method from our earlier publication [14].

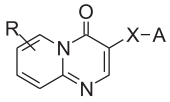
4.1.1. 1-(4-Oxo-4H-pyrido[1,2-a]pyrimidin-3-yl)-3-(2-trifluoromethylbenzyl)urea (*2*)

Compound (**2**) was prepared by refluxing the 3-isocyanato-4*H*-pyrido[1,2-*a*]pyrimidin-4-one [14] (**1**) (0.3 g, 0.16 mmol) with 2-trifluoromethylbenzylamine (0.281 g, 0.16 mmol) in toluene for 6 h. The reaction mixture was cooled to room temp. to get a solid precipitate. The solid precipitate was filtered and washed with hexane. The product was purified by column chromatography using hexane (10%) in ethyl acetate as eluent to get the titled compound (**2**) (0.22 g, 37.8%), m. p. 243-45 °C. R_f 0.52 (AcOEt). IR (KBr): 3315, 3047, 1667, 1638, 1548, 1478, 1328 and 1224 cm^{-1}. ^1H NMR: 4.51–4.52 (d, 2H), 7.25–7.28 (t, 1H), 7.47–7.51 (m, 1H), 7.56–7.64 (m, 3H), 7.68–7.74 (m, 3H), 8.59 (s, 1H), 8.86–8.88 (d, 1H), 9.17 (s, 1H). MS: (*m/z*) 361 (M^+-1).

4.1.2. 1-(4-Oxo-4H-pyrido[1,2-a]pyrimidin-3-yl)-3-(4-trifluoromethylbenzyl)urea (*3*)

3-Isocyanato-4*H*-pyrido[1,2-*a*]pyrimidin-4-one [14] (**1**) (0.3 g, 0.16 mmol) was treated with 4-trifluoromethylbenzylamine (0.281 g, 0.16 mmol) as described above for compound (**2**). The crude product after chromatographic purification using hexane (10%) in ethyl acetate as eluent offered the titled compound (**3**) (0.21 g, 34.4%), m.p. 256–57 °C. R_f 0.48 (AcOEt). IR (KBr): 3328, 3048, 1670, 1636, 1478, 1332, 1222 and 1107 cm^{-1}. ^1H NMR: 4.42–4.44 (d, 2H), 7.25–7.28 (m, 1H), 7.51–7.54 (d, 2H), 7.59–7.64 (m, 2H), 7.69–7.73 (m, 3H), 8.50 (s, 1H), 8.86–8.87 (d, 1H), 9.16 (s, 1H). ^{13}C NMR (DMSO) δ: 155.28, 152.56, 144.29, 144.03, 138.38, 131.89, 127.85, 127.17, 125.97, 125.70, 124.87, 124.83, 120.81, 115.15, 42.50. MS: (*m/z*) 363 (M^++1).

Table 1
Chloroquine sensitive *P. falciparum* (*Pf* 3D7) inhibition data of the title compounds.



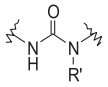
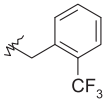
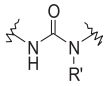
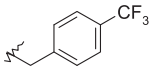
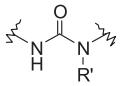
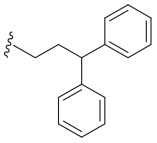
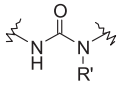
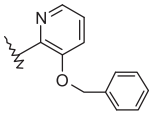
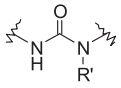
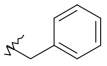
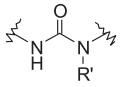
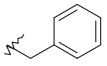
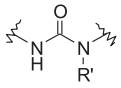
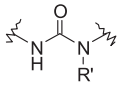
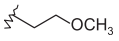
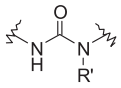
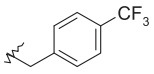
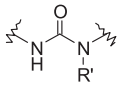
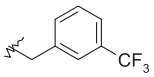
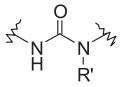
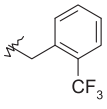
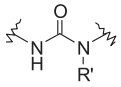
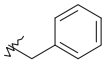
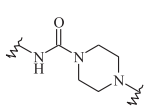
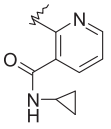
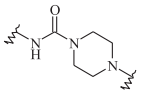
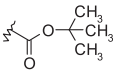
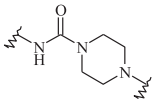
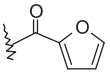
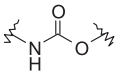
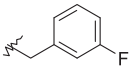
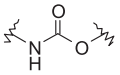
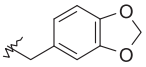
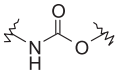
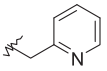
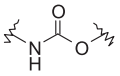
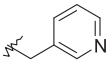
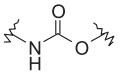
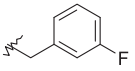
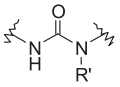
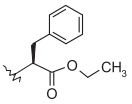
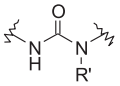
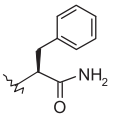
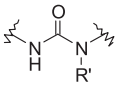
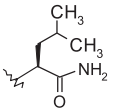
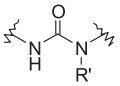
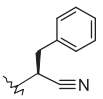
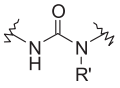
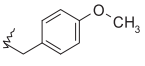
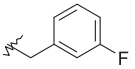
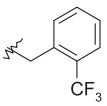
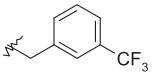
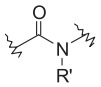
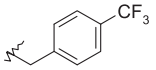
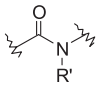
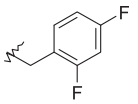
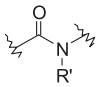
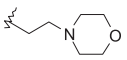
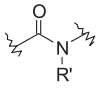
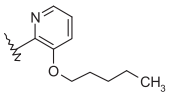
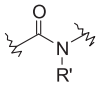
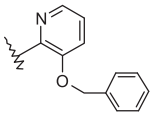
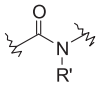
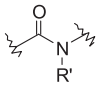
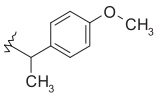
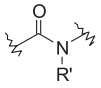
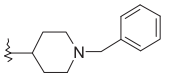
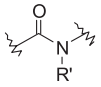
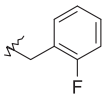
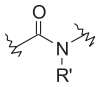
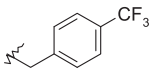
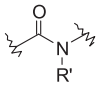
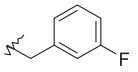
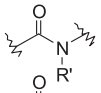
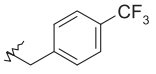
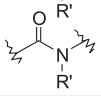
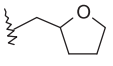
Compound	*R*	*R′*	*X*	*A*	*Pf* 3D7 IC_{50} (μM)
2	H	H			100
3	H	H			>100
5	H	H			>100
7	H	H			>100
9	H				70
10	8-Me				>100
11	7-Cl	H			>100
12	7-Cl	H			>100
13	7-Cl	H			>100
14	7-Cl	H			>100
15	7-Cl	H			>100
16	7-Cl				>100
18	H				>100

Table 1 (*continued*)

Compound	*R*	*R′*	*X*	*A*	*Pf* 3D7 IC_{50} (μM)
19	7-Cl				>100
20	7-Cl				>100
21	H				33
22	H				>100
23	7-Cl				>100
24	7-Cl				>100
25	7-Cl				>100
26	H	H			>100
28	H	H			>100
29	H	H			>100
30	H	H			>100
31	H	H			>100
33	H	H			>100
34	H	H			43
35	H	H			>100
36	H	H			65

(*continued on next page*)

Table 1 (*continued*)

Compound	*R*	*R′*	*X*	*A*	*Pf* 3D7 IC_{50} (μM)
37	H	H			37
38	H	H			59
39	H	H			>100
40	H	H			>100
41	H	H			100
43	H	H			>100
44	H	H			>100
45	H	H			>100
46	H				>100
47	8-Me	H			70
53	7-(4-MeO-Ph)-	H			>100
55	7-(4-MeO-Ph)-	H			>100
56	7-(4-MeO-Ph)-	H			100

*4.1.3. 1-(2,4-Difluorobenzyl)-3-(4-oxo-4H-pyrido[1,2-a]pyrimidin-3-yl)urea (**4**)*

The coupling reaction of the 3-isocyanato-4*H*-pyrido[1,2-*a*]pyrimidin-4-one [14] (**1**) (0.3 g, 0.16 mmol) was done with 2,4-difluorobenzylamine (0.229 g, 0.16 mmol) as described above for compound (**2**). Work-up of the reaction mixture followed by chromatographic purification of the crude product using hexane (5%) in ethyl acetate as eluent yielded compound (**4**) (0.1 g, 18.8%), m.p. 170–71 °C. R_f 0.48 (AcOEt). IR (KBr): 3328, 1676, 1558, 1483, 1236 and 1127 cm^{-1}. ^1H NMR: 4.35–4.37 (d, 2H), 7.14–7.20 (m, 2H), 7.24–7.28 (m, 2H), 7.52–7.55 (t, 1H), 7.62–7.64 (d, 1H), 7.69–7.74 (m, 1H), 8.50 (s, 1H), 8.85–8.87 (d, 1H), 9.14 (s, 1H). MS: (*m/z*) 331 (M^++1). Anal. Calcd. for $C_{16}H_{12}F_2N_4O_2$: C, 58.18; H, 3.66; N,16.96. Found: C, 58.34; H, 3.78; N, 16.85.

*4.1.4. 1-(3,3-Diphenyl)propyl-3-(4-oxo-4H-pyrido[1,2-a]pyrimidin-3-yl)urea (**5**)*

3-Isocyanato-4*H*-pyrido[1,2-*a*]pyrimidin-4-one [14] (**1**) (0.25 g, 0.13 mmol) was reacted with 3,3-diphenylpropylamine (0.282 g, 0.13 mmol) as described above for compound (**2**). The chromatographic purification of the crude product using hexane (10%) in ethyl acetate as eluent afforded the urea derivative (**5**) (0.21 g,

Table 2
Selectivity index of moderately active compounds on human cell line (Huh7).

Compound	IC_{50} (μM)		Selectivity index (IC_{50} Huh7/IC_{50} *pf* 3D7)
	Pf 3D7	Huh7	
21	33	>100	>3
36	65	>200	>3
37	37	>200	>5.4
38	59	>200	>3.3
47	70	>100	>1.4

39.4%), m.p. 259–60 °C. R_f 0.62 (AcOEt). IR (KBr): 3324, 3133, 3024, 1664, 1631, 1559, 1476 and 1241 cm^{-1}. ^1H NMR: 2.18–2.23 (m, 2H), 2.97–3.05 (m, 2H), 4.01–4.05 (t, 1H), 7.11–7.34 (m, 12H), 7.60–7.63 (d, 1H), 7.67–7.74 (m, 1H), 8.32 (s, 1H), 8.85–8.92 (dd, 1H), 9.15(s, 1H). ^{13}C NMR (DMSO) δ: 155.20, 152.52, 144.20, 143.79, 138.01, 131.63, 128.14, 127.39, 125.96, 125.86, 125.64, 121.09, 115.07, 47.89, 37.64, 35.16. MS: (*m/z*) 399 (M^++1).

4.1.5. 1-(4-Oxo-4H-pyrido[1,2-a]pyrimidin-3-yl)-3-[3-(n.pentyloxy)pyridin-2-yl]urea (**6**)

Compound (**6**) was prepared by reacting the 3-isocyanato-4*H*-pyrido[1,2-*a*]pyrimidin-4-one [14] (**1**) (0.3 g, 0.16 mmol) with 3-*n*.pentyloxy-2-aminopyridine (0.289 g, 0.16 mmol) as described above for compound (**2**). The crude product after chromatographic purification using methanol (1%) in ethyl acetate as eluent offered the desired product (**6**) (0.2 g, 33.96%), m.p. 178-80 °C. R_f 0.47 (AcOEt). IR (KBr): 3387, 3350, 3067, 1665, 1550, 1478 and 1220 cm^{-1}. ^1H NMR: 0.90–0.94 (t, 3H), 1.32–1.47 (m, 4H), 1.78–1.85 (m, 2H), 4.07–4.11 (t, 2H), 7.05–7.08 (m, 1H), 7.30–7.34 (m, 1H), 7.44–7.46 (d, 1H), 7.67–7.69 (d, 1H), 7.76–7.81 (m, 1H), 7.89–7.91 (dd, 1H), 8.35 (s, 1H), 8.92–8.94 (d, 1H), 9.29 (s, 1H), 12.15 (s, 1H). MS: (*m/z*) 368 (M^++1).

4.1.6. 1-[3-(Benzyloxy)pyridin-2-yl]-3-(4-oxo-4H-pyrido[1,2-a]pyrimidin-3-yl)urea (**7**)

3-Isocyanato-4*H*-pyrido[1,2-*a*]pyrimidin-4-one [14] (**1**) (0.3 g, 0.16 mmol) was treated with 2-amino-3-benzyloxypyridine (0.319 g, 0.16 mmol) as described above for compound (**2**). The crude product was purified by column chromatography using methanol (1%) in ethyl acetate as eluent offering the titled compound (**7**) (0.19 g, 30.6%), m.p. 249–-50 °C. R_f 0.46 (AcOEt). IR (KBr): 3133, 1675, 1558, 1482, 1192 and 1118 cm^{-1}. ^1H NMR: 5.29 (s, 2H), 7.07 (m, 1H), 7.33–7.42 (m, 3H), 7.51–7.57 (m, 3H), 7.70–7.76 (m, 1H), 7.77–7.79 (m, 1H), 7.91–7.92 (d, 1H), 8.46 (s, 1H), 8.90–8.94 (m, 1H), 9.22–9.28 (d, 1H), 9.52 (s, 1H), 12.06 (s, 1H). MS: (*m/z*) 388 (M^++1).

4.1.7. 1-[6-(Morpholin-4-yl)pyridin-2-yl]-3-(4-oxo-4H-pyrido[1,2-a]pyrimidin-3-yl)urea (**8**)

Compound (**8**) was prepared by treating the 3-isocyanato-4*H*-pyrido[1,2-*a*]pyrimidin-4-one [14] (**1**) (0.3 g, 0.16 mmol) with 2-amino-6-morpholinopyridine (0.287 g, 0.16 mmol) as described above for compound (**2**). The chromatographic purification of the crude product using methanol (1%) in ethyl acetate as eluent offered compound (**8**) (0.25 g, 42.5%), m.p. 275–76 °C. R_f 0.45 (AcOEt). IR (KBr): 3118, 1688, 1558, 1484, 1440, 1229 and 1113 cm^{-1}. ^1H NMR: 3.66–3.68 (b, 4H), 3.95–3.97 (b, 4H), 6.17–6.20 (d, 1H), 6.29–6.31 (d, 1H), 7.11–7.15 (m, 1H), 7.48–7.52 (m, 2H), 7.58–7.62 (m, 1H), 7.67–7.70 (d, 1H), 8.97–8.99 (d, 1H), 9.53 (s, 1H), 11.48 (s, 1H). MS: (*m/z*) 367 (M^++1).

4.1.8. 1-Benzyl-1-isopropyl-3-(4-oxo-4H-pyrido[1,2-a]pyrimidin-3-yl)urea (**9**)

3-isocyanato-4*H*-pyrido[1,2-*a*]pyrimidin-4-one [14] (**1**) (0.25 g, 0.13 mmol) and *N*-isopropyl benzylamine (0.199 g, 0.13 mmol) were reacted as described above for compound (**2**). The crude product by chromatographic purification using hexane (10%) in ethyl acetate offered the titled compound (**9**) (0.15 g, 30.6%), m.p. 145–46 °C. R_f 0.61 (AcOEt). IR (KBr): 3373, 1641, 1527, 1483, 1410, 1232, 1176, and 940 cm^{-1}. ^1H NMR: 1.14–1.16 (d, 6H), 4.51–4.52 (m, 1H), 4.55 (s, 2H), 7.25–7.29 (m, 2H), 7.36–7.37 (d, 4H), 7.50 (s, 1H), 7.63–7.65 (d, 1H), 7.73–7.77 (m, 1H), 8.78–8.80 (d, 1H), 8.96 (s, 1H). MS: (*m/z*) 337 (M^+−1).

4.1.9. 1-Benzyl-1-isopropyl-3-(8-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidin-3-yl)urea (**10**)

3-Isocyanato-8-methyl-4*H*-pyrido[1,2-*a*]pyrimidin-4-one [14] (**1**) (0.25 g, 0.12 mmol) in toluene was refluxed with *N*-isopropyl benzylamine (0.185 g, 0.12 mmol) for 4 h to get a solid. The solid so obtained were reacted as described above for compound (**2**). Chromatographic purification of the crude product using hexane (5%) in ethyl acetate provided the titled compound (**10**) (0.16 g, 36.7%), m.p. 161–62 °C. R_f 0.62 (AcOEt). IR (KBr): 3353, 3031, 2971, 1642, 1546, 1486, 1403 and 1242 cm^{-1}. ^1H NMR: 1.13–1.15 (d, 6H), 2.41 (s, 3H), 4.49–4.51 (m, 1H), 4.54 (s, 2H), 7.13–7.15 (d, 1H), 7.25–7.26 (m, 1H), 7.35–7.36 (d, 4H), 7.45 (b, 2H), 8.70–8.72 (d, 1H), 8.87 (s, 1H). ^{13}C NMR ($CDCl_3$) δ: 155.41, 153.58, 144.89, 143.85, 140.47, 137.87, 128.90, 127.52, 126.45, 125.49, 124.49, 120.10, 118.15, 47.00, 45.76, 21.23, 20.82. MS: (*m/z*) 349 (M^+), 350.9 (M^++2). Anal. Calcd. for $C_{20}H_{22}N_4O_2$: C, 68.55; H, 6.33; N, 15.99. Found: C, 68.76; H, 6.54; N, 15.89.

4.1.10. 1-(7-Chloro-4-oxo-4H-pyrido[1,2-a]pyrimidin-3-yl)-3-(thiophen-2-yl)methylurea (**11**)

7-Chloro-3-isocyanato-4*H*-pyrido[1,2-*a*]pyrimidin-4-one [14] (**1**) (0.25 g, 0.11 mmol) in toluene was refluxed with (2-thiophenyl)methylamine (0.127 g, 0.11 mmol) to get a solid. The solid so obtained was filtered and washed with hexane. The crude product was purified by column chromatography using 1% methanol in ethyl acetate to obtain the compound (**11**) (0.15 g, 39.76%), m.p. 285–87 °C. R_f 0.6 (AcOEt). IR (KBr): 3272, 3024, 1652, 1562, 1472, 1337, 1240, and 1155 cm^{-1}. ^1H NMR: 4.49–4.50 (d, 2H), 6.97–6.98 (m, 1H), 7.00 (s, 1H), 7.40–7.41 (d, 1H), 7.56 (b, 1H), 7.64–7.66 (d, 1H), 7.72–7.74 (d, 1H), 8.51 (s, 1H), 8.89 (s, 1H), 9.18 (s, 1H). ^{13}C NMR ($CDCl_3$) δ: 159.19, 153.94, 152.81, 147.57, 144.08, 143.05, 139.60, 133.71, 127.73, 124.54, 123.79, 120.97, 117.26, 111.55, 43.93. MS: (*m/z*) 335.34(M^++1), 337.38 (M^++2).

4.1.11. 1-(7-Chloro-4-oxo-4H-pyrido[1,2-a]pyrimidin-3-yl)-3-(2-methoxyethyl)urea (**12**)

A mixture of 7-Chloro-3-isocyanato-4*H*-pyrido[1,2-*a*]pyrimidin-4-one [14] (**1**) (0.25 g, 0.11 mmol), 2-methoxyethylamine (0.162 g, 0.11 mmol) and toluene refluxed for 4 h to get solid ppt. The solid so obtained was filtered and washed with hexane. Chromatographic purification of the product using hexane (15%) in ethyl acetate accomplished the desired compound (**12**) (0.22 g, 65.74%), m.p. 254–56 °C. R_f 0.65 (5% MeOH in AcOEt). IR (KBr): 3370, 3116, 2972, 1702, 1649, 1557, 1528, 1434, 1354, 1229, 1199, 1106, 817, 757 and 593 cm^{-1}. ^1H NMR: 3.24–3.39 (m, 7H), 7.19–7.21 (m, 1H), 7.63–7.65 (d, 1H), 7.70–7.73 (dd, 1H), 8.51 (s, 1H), 8.83–8.84 (b, 1H), 9.16 (s, 1H). MS: (*m/z*) 297.39 (M^+), 299.38 (M^++2).

4.1.12. 1-(7-Chloro-4-oxo-4H-pyrido[1,2-a]pyrimidin-3-yl)-3-(4-trifluoromethyl)benzylurea (**13**)

A mixture of 7-chloro-3-isocyanato-4*H*-pyrido[1,2-*a*]pyrimidin-4-one [14] (**1**) (0.25 g, 0.11 mmol) and 4-trifluoromethylbenzylamine (0.197 g, 0.11 mmol) in toluene was refluxed for 4 h. Solid precipitate so obtained was filtered and washed with hexane. Chromatographic purification of the crude product using 1% methanol in ethyl acetate yielded the titled compound (**13**) (0.23 g, (51.4%), m.p. 287–89 °C. R_f 0.61 (1% MeOH in AcOEt). IR (KBr): 3397, 3187, 1692, 1656, 1632, 1364

and 1226 cm^{-1}. ^1H NMR: 4.42–4.44 (d, 2H), 7.57–7.66 (m, 6H), 7.72–7.75 (dd, 1H), 8.58 (s, 1H), 8.84–8.85 (d, 1H), 9.16 (s, 1H). MS: (*m/z*) 397.34(M^+), 399.38 (M^++2).

4.1.13. 1-(7-Chloro-4-oxo-4H-pyrido[1,2-a]pyrimidin-3-yl)-3-(3-trifluoromethyl)benzylurea (**14**)

7-Chloro-3-isocyanato-4*H*-pyrido[1,2-*a*]pyrimidin-4-one [14] (**1**) (0.25 g, 0.11 mmol),3-trifluoromethylbenzylamine (0.197 g, 0.11 mmol) and toluene (50 ml) was refluxed for 4 h. The solid ppt so obtained was filtered and washed with hexane. The crude product was purified by column chromatography using hexane (5%) in ethyl acetate to furnish the titled compound (**14**) (0.22 g, 49.1%), m.p. 260–62 °C. R_f 0.6 (1% MeOH in AcOEt). IR (KBr): 3312, 3282, 3085, 1677, 1646, 1564, 1488, 1433, 1331, 1243, 1160, 1107, 1016, 821, 765 and 644 cm^{-1}. ^1H NMR: 4.42–4.43 (d, 2H), 7.51–7.53 (d, 2H), 7.61–7.63 (m, 2H), 7.70–7.75 (m, 3H), 8.60 (s, 1H), 8.84–8.85 (d, 1H), 9.17 (s, 1H). MS: (*m/z*) 397.34(M^+), 399.32 (M^++2).

4.1.14. 1-(7-Chloro-4-oxo-4H-pyrido[1,2-a]pyrimidin-3-yl)-3-(2-trifluoromethyl)benzylurea (**15**)

7-Chloro-3-isocyanato-4*H*-pyrido[1,2-*a*]pyrimidin-4-one [14] (**1**) (0.25 g, 0.11 mmol) in toluene was refluxed with 2-trifluoromethylbenzylamine (0.197 g, 0.11 mmol) to get solid precipitate. The solid so obtained was filtered and washed with hexane. Chromatographic purification of the crude product using hexane (5%) in ethyl acetate furnished the titled compound (**15**) (0.23 g, 51.4%), m.p. 276–78 °C. R_f 0.62 (1% MeOH in AcOEt). IR (KBr): 3362, 3126, 1707, 1622, 1558, 1461, 1311 and 1225 cm^{-1}. ^1H NMR: 4.51–4.52 (d, 2H), 7.47–7.51 (m, 1H), 7.59–7.75 (m, 6H), 8.68 (s, 1H), 8.85 (b, 1H), 9.17 (s, 1H). ^{13}C NMR (DMSO) δ: 155.36, 152.10, 142.76, 138.32, 137.92, 133.49, 132.97, 129.25, 128.73, 127.85, 127.65, 126.54, 126.01, 125.96, 123.72, 123.24, 121.55. MS: (*m/z*) 397.34 (M^+), 399.32, (M^++2).

4.1.15. 1-Benzyl-3-(7-chloro-4-oxo-4H-pyrido[1,2-a]pyrimidin-3-yl)-1-isopropylurea (**16**)

7-Chloro-3-isocyanato-4*H*-pyrido[1,2-*a*]pyrimidin-4-one [14] (**1**) (0.25 g, 0.11 mmol) in toluene (25 ml) was refluxed with *N*-isopropylbenzylamine (0.168 g, 0.11 mmol) for 5 h to get solid precipitate. Chromatographic purification of the crude product using hexane (10%) in ethyl acetate offered the desired compound (**16**) (0.2 g, 47.8%), m.p. 158–60 °C. R_f 0.64 (AcOEt). IR (KBr): 3447, 1663, 1630, 1521, 1487, 1234, 1114 and 1070 cm^{-1}. MS: (*m/z*) 371.42 (M^+), 373.41(M^++2). Anal. Calcd. for $C_{19}H_{19}ClN_4O_2$: C, 61.54; H, 5.16; N, 15.11. Found: C, 61.68; H, 5.28; N, 15.25.

4.1.16. 1-Benzyl-3-(7-chloro-4-oxo-4H-pyrido[1,2-a]pyrimidin-3-yl)-1-phenethylurea (**17**)

Reaction of 7-chloro-3-isocyanato-4*H*-pyrido[1,2-*a*]pyrimidin-4-one [14] (**1**) (0.25 g, 0.11 mmol) and *N*-benzyl phenylethylamine (0.238 g, 0.11 mmol) was done as described above for compound (**2**). The crude product was purified by column chromatography using 2% methanol in ethyl acetate to obtain the titled compound (**17**) (0.18 g, 36.8%), m.p. 195–97 °C. R_f 0.55 (1% MeOH in AcOEt). IR (KBr): 3345, 3028, 1647, 1550, 1493, 1435, 1366 and 1233 cm^{-1}. ^1H NMR: 2.85–2.89 (t, 2H), 3.35–3.59 (t, 2H), 4.58 (s, 2H), 7.14–7.38 (m, 11H), 7.68–7.71 (d, 1H), 7.81–7.82 (b, 2H), 8.88 (b, 1H), 8.92 (s, 1H). MS: (*m/z*) 433.42 (M^+), 435.41 (M^++2).

4.1.17. N-(4-Oxo-4H-pyrido[1,2-a]pyrimidin-3-yl)-4-[3-(N-cyclopropylcarboxamido)pyridin-2-yl] piperazinecarboxamide (**18**)

3-Isocyanato-4*H*-pyrido[1,2-*a*]pyrimidin-4-one [14] (**1**) (0.3 g, 0.16 mmol) in toluene (25 ml) was refluxed with *N*-cyclopropyl-2-(piperazine-1-yl)pyridine-3-carboxamide (0.319 g, 0.16 mmol) to get a solid precipitate. The crude compound was purified by column chromatography using methanol (3%) in ethyl acetate as eluent to get the desired compound (**18**) (0.22 g, 31.6%), m.p. 140–42 °C. R_f 0.4 (AcOEt). IR (KBr): 3296, 3035, 1654, 1530, 1484, 1434, 1234 and 1134 cm^{-1}. ^1H NMR: 0.55–0.58 (m, 2H), 0.69–0.72 (m, 2H), 2.82–2.83 (m, 1H), 3.31–3.36 (t, 4H), 3.57–3.59 (t, 4H), 6.89–6.92 (m, 1H), 7.32–7.36 (m, 1H), 7.65–7.67 (m, 2H), 7.81–7.85 (m, 1H), 8.08 (s, 1H), 8.23–8.25 (dd, 1H), 8.51–8.53 (d, 1H), 8.76 (s, 1H), 8.91–8.93 (d, 1H). ^{13}C NMR (DMSO) δ: 168.35, 157.59, 154.70, 153.50, 148.34, 145.73, 142.89, 138.18, 133.52, 126.13, 126.01, 120.79, 119.17, 115.76, 115.46, 48.47, 43.43, 22.59, 5.61. MS: (*m/z*) 434 (M^++1).

4.1.18. N-[(7-Chloro-4-oxo-4H-pyrido[1,2-a]pyrimidin-3-yl)-(4-tert-butyloxycarbonyl)piperazine]-1-carboxamide (**19**)

7-Chloro-3-isocyanato-4*H*-pyrido[1,2-*a*]pyrimidin-4-one [14] (**1**) (0.25 g, 11 mmol) was reacted with boc-piperazine (0.209 g, 0.11 mmol) as described above for compound (**17**). Chromatographic purification of the crude product using hexane (10%) in ethyl acetate provided the desired compound (**19**) (0.2 g, 43.4%), m.p. 229–30 °C. R_f 0.6 (1% MeOH in AcOEt). IR (KBr): 3393, 1691, 1650, 1629, 1490, 1422, 1233 and 1173 cm^{-1}. ^1H NMR: 1.42 (s, 9H), 3.39 (b, 4H), 3.44–3.45 (b, 4H), 7.68–7.71 (d, 1H), 7.83–7.85 (d, 1H), 8.13 (s, 1H), 8.78 (s, 1H), 8.88–8.89 (d, 1H). MS: (*m/z*) 408.41, 410.39 (M^+−1).

4.1.19. N-[(7-Chloro-4-oxo-4H-pyrido[1,2-a]pyrimidin-3-yl)-4-(furan-2-carbonyl)piperazine]-1-carboxamide (**20**)

7-Chloro-3-isocyanato-4*H*-pyrido[1,2-*a*]pyrimidin-4-one [14] (**1**) (0.25 g, 0.11 mmol) was reacted with 1-(furan-2-carbonyl) piperazine (0.203 g, 0.11 mmol) as described above for compound (**18**). Chromatographic purification of the crude product using 1% methanol in ethyl acetate offered the desired compound (**20**) (0.19 g, 41.9%), m.p. 227–28 °C. R_f 0.58 (1% MeOH in AcOEt). IR (KBr): 3446, 3086, 1680, 1626, 1427, 1290, 1242 and 1199 cm^{-1}. ^1H NMR: 3.57–3.58 (b, 4H), 3.74 (b, 4H), 6.65 (s, 1H), 7.04–7.05 (d, 1H), 7.69–7.71 (d, 1H), 7.83–7.86 (m, 1H), 7.87 (s, 1H), 8.18 (s, 1H), 8.80 (s, 1H), 8.89 (b, 1H). MS: (*m/z*) 402 (M^+−1).

Anal. Calcd. for $C_{18}H_{16}ClN_5O_4$: C, 53.81; H, 4.01; N, 17.43. Found: C, 58.97; H, 4.17; N, 17.57.

4.1.20. 3-Fluorobenzyl (4-oxo-4H-pyrido[1,2-a]pyrimidin-3-yl) carbamate (**21**)

A mixture of 3-isocyanato-4*H*-pyrido[1,2-*a*]pyrimidin-4-one [14] (**1**) (0.25 g, 0.13 mmol) and 3-fluorobenzyl alcohol (0.169 g, 0.13 mmol) were refluxed together in toluene. The crude product was purified by column chromatography using hexane (20%) in ethyl acetate to get the titled compound (**21**) (0.11 g, 26.2%), m.p. 158–60 °C. R_f 0.65 (AcOEt). IR (KBr): 3416, 3264, 1716, 1659, 1480, 1441, 1403, 1234, 1200, 1184, 1134, 796 and 773 cm^{-1}. ^1H NMR: 5.19 (s, 2H), 7.14–7.19 (m, 1H), 7.27–7.37 (m, 3H), 7.41–7.47 (m, 1H), 7.69–7.71 (d, 1H), 7.85–7.89 (m, 1H), 8.72 (s, 1H), 8.93–8.95 (d, 1H), 9.16 (b, 1H). MS: (*m/z*) 314, (M^+−1).

4.1.21. 3,4-Methylenedioxybenzyl(4-oxo-4H-pyrido[1,2-a] pyrimdin-3-yl)carbamate (**22**)

3-Isocyanato-4*H*-pyrido[1,2-*a*]pyrimidin-4-one [14] (**1**) (0.5 g, 0.26 mmol) was reacted with 3,4-methylenedioxybenzyl alcohol (0.244 g, 0.106 mmol) as described above for compound (**21**). The chromatographic purification of the crude product using hexane (10%) in ethyl acetate as eluent offered the titled compound (**22**) (0.18 g, 33%), m. p. 175–76 °C. R_f 0.5 (AcOEt). IR (KBr): 3242, 3031, 1717, 1666, 1485, 1443, 1235 and 1137 cm^{-1}. ^1H NMR: 5.06 (s, 2H), 6.02 (s, 2H), 6.92 (s, 2H), 7.02 (s, 1H), 7.33–7.37 (m, 1H), 7.69–7.71 (d, 1H), 7.84–7.89 (m, 1H), 8.70 (b, 1H), 8.92–8.94 (d, 1H), 9.01 (b, 1H). ^{13}C NMR (DMSO) δ: 153.60, 152.96, 147.29, 147.06, 146.12,

133.77, 129.67, 126.19, 126.00, 121.64, 117.94, 115.67, 108.50, 107.75, 100.78, 66.27, 52.00. MS: (*m/z*) 340 (M^++1).

4.1.22. 2-Pyridinylmethyl(7-chloro-4-oxo-4H-pyrido[1,2-a] pyrimidin-3-yl)carbamate (**23**)

7-Chloro-3-isocyanato-4*H*-pyrido[1,2-*a*]pyrimidin-4-one [14] (**1**) (0.25 g, 0.11 mmol) was reacted with pyridine-2-methanol (0.176 g, 0.135 mmol) as described above for compound (**21**). The crude product was purified by column chromatography using 2% methanol in ethyl acetate to accomplish the titled compound (**23**) (0.05 g, 13.4%), m.p. 196–97 °C. R_f 0.62 (2% MeOH in AcOEt). IR (KBr): 3451, 3245, 1724, 1652, 1477, 1354, 1236 and 694 cm^{-1}. ^1H NMR: 5.23 (s, 2H), 7.33–7.36 (d, 1H), 7.51–7.53 (d, 1H), 7.71–7.73 (d, 1H), 7.83–7.87 (t, 1H), 7.88–7.91 (dd, 1H), 8.55–8.56 (d, 1H), 8.76 (s, 1H), 8.92–8.93 (d, 1H), 9.32 (s, 1H). MS: (*m/z*) 331.37, 333.35 (M^+−1).

4.1.23. 3-Pyridinylmethyl(7-chloro-4-oxo-4H-pyrido[1,2-a] pyrimidin-3-yl)carbamate (**24**)

7-Chloro-3-isocyanato-4*H*-pyrido[1,2-*a*]pyrimidin-4-one [14] (**1**) (0.25 g, 0.11 mmol) and pyridine-3-methanol (0.123 g, 0.11 mmol) were reacted as described above for compound (**21**) to get solid precipitate. The solid so obtained was filtered and washed with hexane. Chromatographic purification of the crude product using hexane (5%) in ethyl acetate provided the intended compound (**24**) (0.15 g, 40.2%), m.p. 293–95 °C. R_f 0.65 (AcOEt). IR (KBr): 3415, 3251, 1722, 1664, 1630, 1402 and 1232 cm^{-1}. ^1H NMR: 5.22 (s, 2H), 7.41–7.45 (m, 1H), 7.70–7.73 (d, 1H), 7.86–7.91 (m, 2H), 8.54–8.55 (d, 1H), 8.66 (b, 1H), 8.74 (b, 1H), 8.91–8.92 (d, 1H) 9.16 (s, 1H). MS: (*m/z*) 331.37, 333.35, (M^+−1).

4.1.24. 3-Fluorobenzyl(7-chloro-4-oxo-4H-pyrido[1,2-a]pyrimidin-3-yl)carbamate (**25**)

Compound (**25**) was obtained by reaction of 7-Chloro-3-isocyanato-4*H*-pyrido[1,2-*a*]pyrimidin-4-one [14] (**1**) (0.25 g, 0.11 mmol) with 3-fluorobenzyl alcohol (0.142 g, 0.11 mmol) to get solid precipitate. The solid so obtained was filtered and washed with hexane and purified by column chromatography using hexane (10%) in ethyl acetate to give the titled compound (**25**) (0.06 g, 15.3%), m.p. 240–42 °C. R_f 0.62 (1% MeOH in AcOEt). IR (KBr): 3431, 3276, 1726, 1660, 1546, 1229 and 1127 cm^{-1}. ^1H NMR: 5.15 (s, 2H), 7.20–7.24 (m, 2H), 7.48–7.51 (m, 2H), 7.70–7.72 (d, 1H), 7.88–7.90 (d, 1H), 8.74 (s, 1H), 8.91 (s, 1H), 9.18 (s, 1H). MS: (*m/z*) 350 (M^+−1), 348 (M^+−3). Anal. Calcd. for $C_{16}H_{11}ClFN_3O_3$: C, 55.27; H, 3.19; N, 12.08. Found: C, 55.42; H, 3.31; N, 12.19.

4.1.25. Ethyl (2S)-2-{[(4-oxo-4H-pyrido[1,2-a]pyrimidin-3-yl) carbamoyl]amino}-3-phenylpropanoate (26)

3-Isocyanato-4*H*-pyrido[1,2-*a*]pyrimidin-4-one [14] (**1**) (1.94 g, 6.1 mmol) and L-phenylalanine ethyl ester (2 g, 6.1 mmol) in toluene (25 ml) were heated together at 80 °C for 6 h. The reaction mixture was cooled to RT to yield a solid precipitate. The solid precipitate was filtered, washed with hexane and dried to get the titled compound (**26**) (2 g, 62.5%), m. p. 158–59 °C. R_f 0.75 (AcOEt). IR (KBr): 3338, 3032, 1733, 1688, 1629, 1132 and 1185 cm^{-1}. MS: 381(M^++1).

4.1.26. Methyl (2S)-4-methyl-2-{[(4-oxo-4H-pyrido[1,2-a] pyrimidin-3-yl)carbamoyl]amino} pentanoate (**27**)

Compound (**27**) was obtained by reacting 3-isocyanato-4*H*-pyrido[1,2-*a*]pyrimidin-4-one [14] (**1**) (2.85 g, 1.37 mmol) with L-leucine methyl ester (2 g, 1.37 mmol) as described above for compound (**26**). The crude product was recrystallized from methanol to yield compound (**27**) (2.1 g, 45.8%), m.p. 129–30 °C. R_f 0.78 (AcOEt). IR (KBr): 3325, 3276, 1745, 1657, 1634, 1550, 1528 and 1191 cm^{-1}. MS: (*m/z*) 333 (M^++1).

4.1.27. (2S)-2-{[(4-Oxo-4H-pyrido[1,2-a]pyrimidin-3-yl) carbamoyl]amino}-3-phenylpropanamide (**28**)

A mixture of compound (**26**) (2 g, 0.52 mmol) and liq. ammonia was stirred for 12 h. The solid precipitate so obtained was filtered, washed with water and recrystallized from methanol to furnish compound (**28**) (1.8 g, 97.4%), m. p. 239–40 °C. R_f 0.35 (AcOEt). IR (KBr): 3329, 1634, 1519, 1477, 1244 and 1191 cm^{-1}. MS: (*m/z*) 352 (M^++1).

4.1.28. (2S)-4-Methyl-2-{[(4-oxo-4H-pyrido[1,2-a]pyrimidin-3-yl) carbamoyl]amino}pentanamide (**29**)

Compound (**27**) (2 g, 0.52 mmol) was stirred in liq. ammonia for 12 h to get the solid precipitate. The precipitated recrystallized from methanol to obtain compound (**29**) (1.6 g 83.7%), m. p. 243–44 °C. R_f 0.4 (AcOEt). IR (KBr): 3361, 3320, 1672, 1628, 1528, 1469, 1437 and 1234 cm^{-1}. ^{13}C NMR (DMSO) δ: 154.31, 152.88, 145.02, 139.39, 135.64, 133.60, 129.65, 128.64, 127.38, 126.41, 119.92, 116.21, 42.80, 37.71. MS: (*m/z*) 318 (M^++1).

4.1.29. 1-[(1S)-1-(Cyano-2-phenyl)ethyl]-3-(4-oxo-4H-pyrido[1,2-a]pyrimidin-3-yl)urea (**30**)

The amide (**28**) (1.2 g, 0.34 mmol) was dissolved in dimethylformamide (2.5 ml) and cooled to 0 °C. Cyanuric chloride (1.26 g, 0.68 mmol) was added in small portions and stirred for 2 h. The reaction mixture was quenched in ice-cold water (20 ml) to get solid precipitate. The solid precipitate was filtered and dried. The crude product so obtained was purified by column chromatography to earn the compound (**30**) (0.34 g, 29.8%), m.p. 223–25 °C. R_f 0.45 (AcOEt). IR (KBr): 3303, 2210, 1686, 1631, 1530, 1433, 1227 and 1133 cm^{-1}. ^1H NMR: 3.09–3.19 (m, 2H), 4.92–4.98 (m, 1H), 7.26–7.34 (m, 2H), 7.36–7.37 (m, 4H), 7.63–7.67 (m, 2H), 7.72–7.77 (m, 1H), 8.58 (s, 1H), 8.85–8.87 (d, 1H), 9.10 (s, 1H). ^{13}C NMR ($CDCl_3$) δ: 175.09, 155.00, 153.44, 144.64, 140.88, 132.15, 126.65, 126.59, 120.96, 115.89, 52.37, 51.30, 42.05, 24.81, 22.80, 21.93. MS: (*m/z*) 334 (M^++1).

4.1.30. 1-[(1S) 1-(Cyano-3-methyl)butyl]-3-(4-oxo-4H-pyrido[1,2-a]pyrimidin-3-yl)urea (**31**)

Compound (**30**) was prepared by treating (**29**) (2.0 g, 0.63 mmol) with cyanuric chloride (2.33 g, 1.26 mmol) as described above for compound (**30**). The chromatographic purification of the crude product using hexane (25%) in ethyl acetate as eluent offered compound (**31**) (0.35 g, 18%), m. p. 237–39 °C. R_f 0.55 (AcOEt). IR (KBr): 3342, 2210, 1642, 1557, 1510, 1465, 1434 and 1235 cm^{-1}. ^1H NMR: 0.91–0.96 (d, 6H), 1.64–1.80 (m, 3H), 4.65–4.71(m, 1H), 7.27–7.31 (m, 1H), 7.65–7.67 (d, 2H), 7.73–7.77 (m, 1H), 8.49 (s, 1H), 8.86–8.88 (d, 1H), 9.12 (s, 1H). ^{13}C NMR (DMSO) δ: 154.04, 152.54, 144.48, 138.77, 132.57, 126.00, 125.86, 120.02, 119.64, 115.49, 40.73, 24.27, 21.74, 21.73. MS: (*m/z*) 300 (M^++1). Anal. Calcd. for $C_{14}H_{15}N_5O_2$: C, 58.94; H, 5.30; N, 24.55. Found: C, 58.86; H, 3.42; N, 24.58.

4.1.31. N-(4-Methoxybenzyl)-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxamide (**33**)

In a two neck round bottom flask (100 ml) equipped with calcium chloride guard tube, the acid derivative 4-oxo-4*H*-pyrido[1,2-*a*]-3-carboxylic acid [14] (**32**) (0.5 g, 0.26 mmol) was dissolved in dichloromethane (50 ml) and the solution was cooled to 0 °C. 1-Hydroxybenzotriazole hydrate (0.355 g, 0.26 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (0.603 g, 0.31 mmol) and *N,N*-diisopropylethylamine (0.679 g, 0.52 mmol) were added to the above solution. After 20 min 4-methoxybenzylamine (0.397 g, 0.26 mmol) was added and the reaction mixture was stirred for 1 h

at 0 °C and for another 8 h at room temperature. The reaction mass was quenched by adding cold water (20 ml) and the medium was neutralized using dilute hydrochloric acid, extracted using ethyl acetate (3 × 100 ml), dried over anhydrous sodium sulfate and concentrated to get a dark brown product. The crude product was purified by column chromatography using methanol (2%) in ethyl acetate to offer the compound (**33**) (0.24 g, 28.2%), m. p. 192–94 °C. R_f 0.51 (AcOEt). IR (KBr): 3336, 1679, 1489, 1334, 1297, 1243 and 1030 cm^{-1}. ^1H NMR: 3.80 (s, 3H), 4.62–4.64 (d, 2H), 6.86–6.90 (m, 2H), 7.31–7.33 (d, 2H), 7.34–7.38 (m, 1H), 7.84–7.86 (d, 1H), 7.92–7.97 (m, 1H), 9.19–9.21 (d, 1H), 9.30 (b, 1H), 9.38 (s, 1H). ^{13}C NMR (DMSO) δ: 162.98, 158.31, 157.24, 157.02, 152.07, 139.87, 131.16, 128.79, 128.24, 126.38, 118.41, 113.81, 105.95, 55.03, 41.82. MS: (*m/z*) 310 (M^++1).

4.1.32. *N-(3-Fluorobenzyl)-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxamide (**34**)*

Compound (**34**) was prepared by reacting compound 4-oxo-4*H*-pyrido[1,2-*a*]-3-carboxylic acid [14] (**32**) (0.5 g, 0.26 mmol) with 3-fluorobenzylamine (0.329 g, 0.26 mmol) as described above for compound (**33**). The crude product was purified by column chromatography using hexane (5%) in ethyl acetate as eluent offering the compound (**34**) (0.32 g, 40.9%), m.p. 168–70 °C. R_f 0.64 (AcOEt). IR (KBr): 3327, 3122, 1662, 1623, 1569, 1533, 1500 and 1240 cm^{-1}. ^1H NMR: 4.59–4.61 (d, 2H), 7.06–7.11 (m, 1H), 7.15–7.21 (m, 2H), 7.36–7.41 (m, 1H), 7.59–7.63 (m, 1H), 7.90–7.92 (d, 1H), 8.19–8.23 (m, 1H), 9.05 (s, 1H), 9.19–9.21 (d, 1H), 9.43–9.46 (t, 1H). ^{13}C NMR (DMSO) δ: 163.61, 161.19, 157.42, 152.33, 142.71, 140.14, 130.51, 128.48, 126.57, 123.51, 118.62, 114.28, 114.07, 113.86, 106.06, 42.04. MS: (*m/z*) 298 (M^++1).

4.1.33. *4-Oxo-N-[2-(trifluoromethyl)benzyl]-4H-pyrido[1,2-a]pyrimidine-3-carboxamide (**35**)*

Compound (**35**) was prepared by treating compound 4-oxa-4*H*-pyrido[1,2-*a*]-3-carboxylic acid [14] (**32**) (0.5 g, 0.26 mmol) with 2-trifluoromethylbenzylamine (0.461 g, 0.26 mmol) as described above for compound (**33**). Chromatographic purification of the crude product using hexane (5%) in ethyl acetate as eluent yielded compound (**35**) (0.32 g, 35%), m.p. 158–60 °C. R_f 0.6 (AcOEt). IR (KBr): 3299, 3079, 1695, 1630, 1476, 1315, 1167 and 1094 cm^{-1}. ^1H NMR: 4.76–4.78 (d, 2H), 7.48–7.52 (m, 1H), 7.59–7.69 (m, 3H), 7.75–7.77 (d, 1H), 7.91–7.93 (d, 1H), 8.20–8.24 (m, 1H), 9.05 (s, 1H), 9.21–9.23 (d, 1H), 9.48–9.51 (t, 1H). ^{13}C NMR (DMSO) δ: 163.55, 157.49, 152.36, 140.24, 137.61, 132.96, 129.49, 128.78, 127.74, 126.99, 126.69, 126.00, 125.94, 123.33, 120.61, 118.68, 105.87. MS: (*m/z*) 348 (M^++1).

4.1.34. *4-Oxo-N-[3-(trifluoromethyl)benzyl]-4H-pyrido[1,2-a]pyrimidine-3-carboxamide (**36**)*

4-Oxo-4*H*-pyrido[1,2-*a*]-3-carboxylic acid [14] (**32**) (0.5 g, 0.26 mmol) was reacted with 3-trifluoromethylbenzylamine (0.461 g, 0.26 mmol) as described above for compound (**33**). The chromatographic purification of the crude product using hexane (5%) in ethyl acetate as eluent furnished the compound (**36**) (0.33 g, 36.1%), m.p. 139–40 °C. R_f 0.63 (AcOEt). IR (KBr): 3328, 3113, 1691, 1645, 1532, 1490, 1415 and 1335 cm^{-1}. ^1H NMR: 4.66–4.67 (d, 2H), 7.56–7.63 (m, 3H), 7.66–7.68 (d, 1H), 7.72 (s, 1H), 7.90–7.92 (d, 1H), 8.18–8.23 (m, 1H), 9.05 (s, 1H), 9.20–9.21 (d, 1H), 9.50–9.53 (t, 1H). ^{13}C NMR ($CDCl_3$) δ: 164.01, 158.60, 157.69, 152.48, 139.78, 138.50, 131.02, 130.70, 129.09, 128.06, 127.11, 125.44, 124.34, 122.73, 117.55, 106.83, 42.89. MS: (*m/z*) 348 (M^++1).

4.1.35. *4-Oxo-N-[4-(trifluoromethyl)benzyl]-4H-pyrido[1,2-a]pyrimidine-3-carboxamide (**37**)*

Compound (**37**) was prepared by reacting 4-oxo-4*H*-pyrido[1,2-*a*]-3-carboxylic acid [14] (**32**) (0.5 g, 0.26 mmol) with 4-trifluoromethylbenzylamine (0.461 g, 0.26 mmol) as described above for compound (**33**). The chromatographic purification of the crude product using hexane (5%) in ethyl acetate as eluent yielded compound (**37**) (0.25 g, 26.2%), m. p. 167-69 °C. R_f 0.6 (AcOEt). IR (KBr): 3336, 3115, 1663, 1600, 1570, 1500, 1325 and 1123 cm^{-1}. ^1H NMR: 4.75–4.76 (d, 2H), 7.38–7.41 (m, 1H), 7.49–7.51 (d, 2H), 7.58–7.60 (d, 2H), 7.86–7.88 (d, 1H), 7.96–8.00 (m, 1H), 9.21–9.23 (d, 1H), 9.37–9.38 (d, 1H), 9.47 (b, 1H). ^{13}C NMR ($CDCl_3$) δ: 164.02, 158.60, 157.70, 152.48, 142.82, 138.50, 129.91, 128.95, 127.81, 125.61, 122.80, 120.10, 117.57, 106.81, 42.92. MS: (*m/z*) 348 (M^++1). Anal. Calcd. for $C_{17}H_{12}F_3N_3O_2$: C, 58.79, H,3.48, N, 12.10. Found: C, 58.91; H, 3.62; N, 12.37.

4.1.36. *N-(2,4-Difluorobenzyl)-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxamide (**38**)*

Compound (**38**) was prepared by reacting 4-oxo-4*H*-pyrido[1,2-*a*]-3-carboxylic acid [14] (**32**) (0.5 g, 0.26 mmol) with 2,4-difluoromethylbenzylamine (0.376 g, 0.26 mmol) as described above for compound (**33**). The chromatographic purification of the crude product using hexane (20%) in ethyl acetate as eluent afforded the desired product (**38**) (0.21 g, 25.3%), m. p. 172–75 °C. R_f 0.62 (AcOEt). IR (KBr): 3328, 3125, 1664, 1500, 1430, 1184 and 1072 cm^{-1}. ^1H NMR: 4.60–4.62 (d, 2H), 7.14–7.21 (m, 2H), 7.24–7.29 (m, 1H), 7.59–7.63 (m, 1H), 7.89–7.92 (d, 1H), 8.19–8.23 (m, 1H), 9.03 (s, 1H), 9.20–9.23 (d, 1H), 9.42–9.45 (t, 1H). ^{13}C NMR ($CDCl_3$) δ: 164.03, 159.90, 159.87, 158.56, 157.95, 157.92, 155.54, 152.48, 138.52, 128.09, 127.58, 117.55, 116.44, 114.97, 106.78, 36.99. MS: (*m/z*) 316 (M^++1).

4.1.37. *N-[2-(Morpholin-4-yl)ethyl]-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxamide (**39**)*

Reaction of compound 4-oxo-4*H*-pyrido[1,2-*a*]-3-carboxylic acid [14] (**32**) (0.5 g, 0.26 mmol) with 2-(4-morpholinyl)ethylamine (0.342 g, 0.26 mmol) was effected as described above for compound (**33**). The chromatographic purification of the crude product using methanol (0.2%) in ethyl acetate as eluent offered the titled compound (**39**) (0.18 g, 22.6%), m. p. 193–95 °C. R_f 0.3 (1% MeOH in AcOEt). IR (KBr): 3123, 1690, 1631, 1483, 1400, 1251 and 1115 cm^{-1}. ^1H NMR: 2.43 (b, 4H), 2.50–2.51 (m, 2H), 3.45–3.50 (m, 2H), 3.59–3.61(t, 4H), 7.57–7.61 (m, 1H), 7.84–7.90 (d, 1H), 8.17–8.21 (m, 1H), 9.02 (s, 1H), 9.15–9.16 (t, 1H), 9.20–9.22 (d, 1H). MS: (*m/z*) 303 (M^++1).

4.1.38. *4-Oxo-N-[3-(n.pentyloxy)pyridin-2-yl]-4H-pyrido[1,2-a]pyrimidine-3-carboxamide (**40**)*

Compound (**40**) was prepared by reacting 4-oxo-4*H*-pyrido[1,2-*a*]-3-carboxylic acid [14] (**32**) (0.5 g, 0.26 mmol) with 2-amino-3-*n*.pentyloxypyridine (0.474 g, 0.26 mmol) as described above for compound (**33**). The chromatographic purification of the crude product using methanol (2.5%) in ethyl acetate as eluent produced the titled product (**40**) (0.3 g, 32.3%), m.p. 159–60 °C. R_f 0.3 (AcOEt). IR (KBr): 3292, 3237, 1693, 1595, 1470, 1333 and 1218 cm^{-1}. ^1H NMR: 0.89–0.93 (t, 3H), 1.36–1.43 (m, 2H), 1.49–1.57 (m, 2H), 1.78–1.84 (m, 2H), 4.09–4.13 (t, 2H), 6.55 (s, 1H), 7.13–7.16 (m, 1H), 7.46–7.48 (d, 1H), 7.66–7.70 (m, 1H), 7.95–7.97 (d, 1H), 8.24–8.28 (m, 1H), 9.13 (s, 1H), 9.21–9.24 (d, 1H), 11.51 (s, 1H). ^{13}C NMR ($CDCl_3$) δ: 160.31, 157.96, 152.14, 149.59, 144.25, 141.99, 139.28, 138.46, 127.75, 126.50, 119.20, 117.96, 115.44, 106.56, 68.26, 28.29, 27.49, 21.96, 13.74. MS: (*m/z*) 353 (M^++1).

4.1.39. *N-[3-(Benzyloxy)pyridin-2-yl]-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxamide (**41**)*

4-Oxo-4*H*-pyrido[1,2-*a*]-3-carboxylic acid [14] (**32**) (0.5 g, 0.26 mmol) and 2-amino-3-benzyloxypyridine (0.526 g, 0.26 mmol) were reacted together as described above for compound (**33**). The chromatographic purification of the crude product using methanol

(1%) in ethyl acetate as eluent offered compound (**41**) (0.23 g, 23.4%), m.p. 193–95 °C. R_f 0.55 (AcOEt). IR (KBr): 3480, 3106, 1699, 1598, 1482, 1285 and 1012 cm^{-1}. ^1H NMR: 5.28 (s, 2H), 7.00–7.04 (dd, 1H), 7.23–7.26 (dd, 1H), 7.34–7.38 (m, 1H), 7.41–7.44 (m, 2H), 7.53–7.55 (m, 1H), 7.58–7.60 (d, 2H), 7.88–7.91 (d, 1H), 7.98–8.02 (m, 1H), 8.10–8.14 (m, 1H), 8.16–8.18 (dd, 1H), 9.27–9.31 (m, 1H), 9.49 (s, 1H), 11.76 (s, 1H).^{13}C NMR (DMSO) δ: 165.29, 160.66, 158.44, 157.84, 152.95, 145.05, 141.85, 140.58, 139.35, 136.53, 128.82, 127.97, 127.41, 126.69, 120.64, 119.00, 118.90, 106.37, 69.81. MS: (*m/z*) 373 (M^++1).

4.1.40. *N-[(2,5-Diethoxy)-4-(morpholin-4-yl)phenyl]-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxamide (**42**)*

Compound (**42**) was prepared by treating compound 4-oxo-4*H*-pyrido[1,2-*a*]-3-carboxylic acid [14] (**32**) (0.5 g, 0.26 mmol) with 2,5-diethoxy-4-morpholin-4-yl-phenylamine (0.7 g, 0.26 mmol) as described above for compound (**33**). The chromatographic purification of the crude product using methanol (1%) in ethyl acetate as eluent provided compound (**42**) (0.35 g, 30.3%), m. p. 166–67 °C. R_f 0.7 (AcOEt). IR (KBr): 3263, 1685, 1487, 1260, 1199, 1113, 1043 cm^{-1}. ^1H NMR: 1.44–1.47 (t, 3H), 1.54–1.58 (t, 3H), 3.09–3.10 (t, 4H), 3.89–3.91 (t, 4H), 4.12–4.17 (m, 4H), 6.60 (s, 1H), 7.37–7.41 (m, 1H), 7.85–7.87 (d, 1H), 7.95–7.99 (m, 1H), 8.40 (s, 1H), 9.33–9.34 (d, 1H), 9.42 (s, 1H), 11.49 (s, 1H). MS: (*m/z*) 439 (M^++1).

4.1.41. *N-(3,5-Dimethyl-1,2-oxazol-4-yl)-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxamide (**43**)*

Compound (**43**) was prepared by reacting 4-oxo-4*H*-pyrido[1,2-*a*]-3-carboxylic acid [14] (**32**) (0.5 g, 0.26 mmol) with 3,5-dimethyl-1,2-oxazol-4yl-amine (0.295 g, 0.26 mmol) as described above for compound (**33**). The chromatographic purification of the crude product using hexane (10%) in ethyl acetate as eluent offered compound (**43**) (0.34 g, 45.4%), m.p. 243–45 °C. R_f 0.6 (AcOEt). IR (KBr): 3253, 3209, 1694, 1486, 1332, 1235 and 1067 cm^{-1}. ^1H NMR: 2.15 (s, 3H), 2.33 (s, 3H), 7.65–7.69 (m, 1H), 7.95–7.97 (d, 1H), 8.24–8.29 (m, 1H), 9.09 (s, 1H), 9.26–9.27 (d, 1H), 10.20 (s, 1H). ^{13}C NMR (DMSO) δ: 162.45, 162.12, 157.60, 157.46, 152.27, 139.99, 128.20, 126.52, 118.50, 113.76, 105.44, 99.49, 11.04, 9.49. MS: (*m/z*) 285 (M^++1).

4.1.42. *N-[1-(4-Methoxyphenyl)ethyl]-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxamide (**44**)*

4-Oxo-4*H*-pyrido[1,2-*a*]-3-carboxylic acid [14] (**32**) (0.5 g, 0.26 mmol) was reacted with α-methyl-4-methoxybenzylamine (0.397 g, 0.26 mmol) as described above for compound (**33**) Chromatographic purification of the crude product using methanol (0.2%) in ethyl acetate as eluent afforded compound (**44**) (0.24 g, 28.2%), m.p. 173–75 °C. R_f 0.6 (AcOEt) IR (KBr): 3315, 3128, 1667, 1630, 1506, 1240 and 1025 cm^{-1}. ^1H NMR: 1.61–1.66 (d, 3H), 3.79 (s, 3H), 5.28–5.35 (m, 1H), 6.87–6.91(d, 2H), 7.34–7.38 (m, 3H), 7.83–7.86 (d, 1H), 7.92–7.97 (m, 1H), 9.20–9.22 (d, 1H), 9.33–9.36 (m, 2H). ^{13}C NMR ($CDCl_3$) δ: 162.70, 158.63, 158.39, 157.62, 152.31, 138.27, 135.96, 127.91, 127.30, 127.03, 117.43, 113.98, 107.17, 55.27, 48.36, 22.57. MS: (*m/z*) 324 (M^++1).

4.1.43. *N-(1-Benzylpiperidin-4-yl)-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxamide (**45**)*

Compound (**45**) was obtained by reaction of compound 4-oxo-4*H*-pyrido[1,2-*a*]-3-carboxylic acid [14] (**32**) (0.5 g, 0.26 mmol) with 4-amino-1-benzylpiperidine (0.5 g, 0.26 mmol) as described above for compound (**33**). The chromatographic purification of the crude product using hexane (10%) in ethyl acetate as eluent earned the titled compound (**45**) (0.25 g, 5%), m. p. 180–83 °C. R_f 0.56 (AcOEt). IR (KBr): 3288, 1695, 1544, 1479, 1335 and 1061 cm^{-1}. ^1H NMR: 1.52–1.58 (m, 2H), 1.89–1.92 (d, 2H), 2.18–2.22 (t, 2H), 2.70 (b, 2H), 3.50 (s, 2H), 3.88 (b, 1H), 7.26 (b, 1H), 7.33 (b, 4H), 7.60–7.64 (t, 1H), 7.90–7.92 (d, 1H), 8.19–8.23 (t, 1H), 9.04 (b, 2H), 9.20–9.22 (d, 1H). MS: (*m/z*) 363 (M^++1). Anal. Calcd. for $C_{21}H_{22}N_4O_2$: C, 69.59; H,6.12; N, 15.46. Found: C, 69.72; H, 6.24; N, 15.57.

4.1.44. *N-Cyclopropyl-N-(2-fluorobenzyl)-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxamide (**46**)*

The reaction of 4-oxo-4*H*-pyrido[1,2-*a*]-3-carboxylic acid [14] (**32**) (0.5 g, 0.26 mmol) with *N*-cyclopropyl-2-fluorobenzylamine (0.434 g, 0.26 mmol) was performed as described above for compound (**33**). The chromatographic purification of the crude product using hexane (5%) in ethyl acetate as eluent yielded the desired compound (**46**) (0.24 g, 27%), m.p. 137–40 °C. R_f 0.55 (AcOEt). IR (KBr): 3110, 1669, 1492, 1404, 1090 and 1031 cm^{-1} ^1H NMR: 0.58–0.61 (d, 4H), 3.06 (b, 1H), 4.93 (s, 2H), 7.11–7.13 (m, 1H), 7.23–7.25 (m, 1H), 7.32–7.34 (m, 2H), 7.67 (b, 1H), 7.80–7.82 (d, 1H), 7.90–7.94 (m, 1H), 8.60 (s, 1H), 9.21–9.23 (d, 1H). ^{13}C NMR ($CDCl_3$) δ: 167.90, 162.09, 159.65, 154.40, 153.22, 150.46, 138.44, 129.77, 128.86, 127.73, 125.62, 124.35, 116.04, 115.83, 44.40, 30.25, 14.20, 8.98. MS: (*m/z*) 338 (M^++1).

4.1.45. *N-(4-Trifluorobenzyl)-8-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxamide (**47**)*

Compound (**47**) was prepared by reacting 8-methyl-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidine-3-carboxylic acid [14] (**32**) (0.4 g, 0.19 mmol) with 4-trifluoromethylbenzylamine (0.343 g, 0.19 mmol) as described above for compound (**33**). Chromatographic purification of the crude product using methanol (5%) in methylene chloride as eluent yielded the titled product (**47**) (0.15 g, 21.2%), m.p. 175–77 °C. R_f 0.4 (AcOEt). IR (KBr): 3414, 3294, 1695, 1634, 1545, 1330, 1117, 1015 and 793 cm^{-1}. ^1H NMR: 2.55 (s, 3H), 4.65–4.67 (d, 2H), 7.46–7.49 (dd, 1H), 7.55–7.57 (d, 2H), 7.69–7.73 (m, 3H), 9.00 (s, 1H), 9.08–9.10 (d, 1H), 9.45–9.48 (t, 1H). ^{13}C NMR ($CDCl_3$) δ: 164.24, 158.88, 157.33, 152.33, 151.48, 142.91, 129.55, 129.23, 128.91, 127.81, 125.59, 122.81, 120.15, 105.95, 42.87, 21.66. MS: (*m/z*) 362 (M^++1).

4.1.46. *7-Chloro-4-oxo-N-[4-(trifluoromethyl)benzyl]-4H-pyrido[1,2-a]pyrimidine-3-carboxamide (**48**)*

Compound (**48**) was prepared by reacting 7-chloro-4-oxo-4*H*-pyrido[1,2-*a*]-3-carboxylic acid [14] (**32**) (0.5 g, 0.22 mmol) with 4-trifluoromethylbenzylamine (0.39 g, 0.22 mmol) as described above for compound (**33**). Chromatographic purification of the crude product using hexane (10%) in ethyl acetate as eluent offered compound (**48**) (0.2 g, 23.5%), m.p. 212–14 °C. R_f 0.51 (AcOEt). IR (KBr): 3315, 3091, 1674, 1620, 1532, 1498, 1323 and 1134 cm^{-1}. ^1H NMR: 4.67–4.68 (d, 2H), 7.55–7.57 (d, 2H), 7.70–7.72 (d, 2H), 7.92–7.95 (d, 1H), 8.26–8.29 (dd, 1H), 9.04 (s, 1H), 9.17–9.18 (d, 1H), 9.43–9.46 (t, 1H). MS: (*m/z*) 382.2 (M^++1), 384.2 (M^++2).

4.1.47. *7-Chloro-[N-Cyclopropyl-N-(2-fluorobenzyl)]-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxamide (**49**)*

Compound (**49**) was prepared by reacting the 7-chloro-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidine-3-carboxylic acid [14] (**32**) (0.5 g, 0.22 mmol) with *N*-cyclopropyl-2-fluorobenzylamine (0.367 g, 0.22 mmol) as described above for compound (**33**). The chromatographic purification of the crude product using hexane (10%) in ethyl acetate as eluent provided compound (**49**) (0.28 g, 33.8%), m.p. 209–10 °C. R_f 0.48 (AcOEt). IR (KBr): 3068, 3019, 1688, 1630, 1481, 1344, 1223 and 1030 cm^{-1}. ^1H NMR: 0.51–0.55 (d, 4H), 2.87 (b, 1H), 4.75 (b, 2H), 7.21–7.24 (m, 2H), 7.33–7.36 (m, 1H), 7.63 (b, 1H), 7.84–7.87 (d, 1H), 8.14–8.17 (dd, 1H), 8.47 (s, 1H), 9.14 (s, 1H). MS: (*m/z*) 372.4 (M^++1), 374.3 (M^++2).

*4.1.48. Ethyl 7-(4-methoxyphenyl)-4-oxo-4H-pyrido[1,2-a] pyrimidine-3-carboxylate (**51**)*

Ethyl 7-chloro-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidine-3-carboxylate (**50**) (10 g, 39.6 mmol) was dissolved in dimethoxyethane (300 ml) and 4-methoxyphenylboronic acid (6 g, 39.6 mmol), potassium carbonate (8.194 g, 59.4 mmol) and tetrakis(-triphenylphosphene)palladium(0) (0.046 g, 0.039 mmol) were added to the above reaction mixture under N_2 atmosphere. The reaction mixture was refluxed for 36 h at 90 °C. The reaction mass was filtered and the filtrate was washed with water and extracted using methylene chloride (3 × 100 ml), dried over anhydrous sodium sulfate and concentrated to get a dark brown product. Chromatographic purification of the crude product using ethyl acetate (15%) in hexane as eluent offered the titled compound [(**51**) (3.5 g, 27.3%)], m. p. 166–67 °C. R_f 0.7 (50% AcOEt in hexane). IR (KBr): 3087, 1744, 1609, 1569, 1487, 1296, 1188, 1113, 1045, 826 and 795 cm^{-1}. MS: (*m/z*) 325, (M^+-1).

*4.1.49. 7-(4-Methoxyphenyl)-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (**52**)*

A solution of [(**51**) (5 g, 4.3 mmol)] in conc. HCl (15 ml) was refluxed for 2 h. The reaction mixture was cooled to RT to get a solid precipitate. The solid so obtained was filtered, washed with ether (100 ml) and dried to obtain 7-(4-Methoxyphenyl)-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidine-3-carboxylic acid (**52**) (3.1 g, 99.8%), m.p. 211–12 °C. R_f 0.17 (AcOEt). IR (KBr): 3317, 3087, 2474, 1791, 1772, 1606, 1289 and 1187 cm^{-1}. MS: (*m/z*) 297, (M^+-1).

*4.1.50. N-(3-Fluorobenzyl)-7-(4-methoxyphenyl)-4-oxo-4H-pyrido [1,2-a]pyrimidine-3-carboxamide (**53**)*

Compound 7-(4-Methoxyphenyl)-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidine-3-carboxylic acid (**52**) (0.25 g, 0.08 mmol) and 3-fluorobenzylamine (0.105 g, 0.08 mmol) were reacted as described above for compound (**33**). Chromatographic purification of the crude product using hexane (15%) in ethyl acetate as eluent afforded the titled compound (**53**) (0.16 g, 47.0%), m. p. 171–73 °C. R_f 0.55 (1% MeOH in AcOEt). IR (KBr): 3445, 3319, 3072, 1688, 1611, 1533, 1482, 1336, 1290, 1264, 1184, 1143, 1060, 941, 832, 793, 691, 649 and 556 cm^{-1}. ^{1}H NMR: 3.83 (s, 3H), 4.60–4.62 (d, 2H), 7.07–7.16 (m, 3H), 7.20–7.22 (d, 2H), 7.37–7.42 (d, 1H), 7.79–7.81(d, 2H), 7.95–7.98 (d, 1H), 8.54–8.57 (dd, 1H), 9.04 (s, 1H), 9.27–9.28 (d, 1H), 9.45–9.48 (t, 1H). MS: (*m/z*) 404, (M^+-1). Anal. Calcd. for $C_{23}H_{18}FN_3O_3$: C, 68.48; H, 4.50; N, 10.42. Found: C, 68.66; H, 4.63; N, 10.57.

*4.1.51. N-(3-Trifluoromethylbenzyl)-7-(4-methoxyphenyl)-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxamide (**54**)*

Compound (**52**) (0.3 g, 0.10 mmol) was reacted with 3-trifluoromethylbenzylamine (0.177 g, 0.10 mmol) as described above for compound (**33**). Chromatographic purification of the crude product using hexane (5%) in ethyl acetate as eluent offered the desired compound (**54**) (0.14 g, 30.49%), m.p. 197–98 °C. R_f 0.54 (AcOEt). IR (KBr): 3414, 3290, 3110, 1692, 1563, 1478, 1333, 1262, 1163, 1112, 1066, 828 and 704 cm^{-1}. ^{1}HNMR: 3.84 (s, 3H), 4.68–4.69 (d, 2H), 7.13–7.15 (d, 2H), 7.57–7.59 (d, 2H), 7.71–7.73 (d, 2H), 7.80–7.82 (d, 2H), 7.96–7.99 (d, 1H), 8.55–8.58 (dd, 1H), 9.04 (s, 1H), 9.28–9.29 (d, 1H), 9.52–9.55 (t, 1H). MS: (*m/z*) 454, (M^+-1).

*4.1.52. N-(4-Trifluoromethylbenzyl)-7-(4-methoxyphenyl)-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxamide (**55**)*

Compound (**55**) was obtained by reacting compound (**52**) (0.3 g, 0.10 mmol) with 4-trifluoromethylbenzylamine (0.177 g, 0.10 mmol) as described above for compound (**33**). Chromatographic purification of the crude product using hexane (5%) in ethyl acetate as eluent yielded the titled compound (**55**) (0.16 g, 45.9%), m.p. 209–10 °C. R_f 0.58 (AcOEt). IR (KBr): 3425, 3293, 1689, 1623, 1333, 1263, 792, 583 and 558 cm^{-1}. ^{1}HNMR: 3.83 (s, 3H), 4.67–4.68 (d, 2H), 7.11–7.14 (d, 2H), 7.57–7.64 (m, 2H), 7.67–7.69 (m, 1H), 7.72 (b, 1H), 7.78–7.81 (d, 2H), 7.95–7.97 (d, 1H), 8.53–8.56 (dd, 1H), 9.04 (s, 1H), 9.26 (s, 1H), 9.51–9.54 (t, 1H). MS: (*m/z*) 454, (M^+-1).

*4.1.53. N-(Tetrahydrofuran-2-ylmethyl)-7-(4-methoxyphenyl)-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxamide (**56**)*

Compound [(**52**) (0.25 g, 0.08 mmol)] was reacted with 2-tetrahydrofuranylmethylamine (0.085 g, 0.08 mmol) as described above for compound (**31**). Chromatographic purification of the crude product using hexane (20%) in ethyl acetate as eluent provided compound [(**56**) (0.1 g, 31.2%)], m.p. 176–77 °C. R_f 0.61(1% MeOH in AcOEt). IR (KBr): 3453, 3306, 1689, 1611, 1545, 1337, 1264 and 1180 cm^{-1}. ^{1}H NMR: 1.54–1.61 (m, 1H), 1.80–1.89 (m, 2H), 1.90–1.99 (m, 1H), 3.38–3.39 (d, 1H), 3.52–3.57 (m, 1H), 3.66–3.69 (m, 1H), 3.80–3.81 (d, 1H), 3.84 (s, 3H), 3.96–3.99 (m, 1H), 7.12–7.15 (d, 2H), 7.79–7.84 (d, 2H), 7.91–7.97 (d, 1H), 8.54–8.56 (dd, 1H), 9.03 (s, 1H), 9.17–9.19 (t, 1H), 9.29–9.30 (d, 1H). ^{13}C NMR ($CDCl_3$) δ: 163.95, 160.68, 157.81, 157.60, 151.01, 137.92, 131.16, 128.21, 126.95, 126.88, 124.07, 114.98, 106.95, 77.70, 68.34, 55.46, 43.25, 28.93, 25.98. MS: (*m/z*) 380, (M^+-1). Anal. Calcd. for $C_{21}H_{21}N_3O_4$: C, 66.48; H, 5.58; N, 11.07. Found: C, 66.63; H, 5.72; N, 11.19.

4.2. Measurement of in vitro antimalarial activity of pyrido[1,2-a] pyrimidin-4-one derivatives

The in vitro antimalarial activities of pyrido[1,2-*a*]pyrimidin-4-one derivatives were measured against erythrocytic stages of chloroquine (CQ) sensitive *P. falciparum* strain (*Pf* 3D7). Parasite strains were cultivated by the method of Trager and Jensen [15], with minor modifications. Parasite cultures were maintained in fresh O^+ human erythrocytes at 4% hematocrit in complete medium (RPMI1640 with 0.2% sodium bicarbonate, 0.5% Albumax, 45 mg l^{-1} hypoxanthine, and 50 mg l^{-1} gentamicin) at 37 °C under reduced O_2 (gas mixture contains 5% O_2, 5% CO_2, and 90% N_2). Stock solutions of CQ were prepared in water (Milli-Q grade) and pyrido [1,2-*a*]pyrimidin-4-one derivatives (stock concentrations 50 mM) were dissolved in dimethyl sulfoxide (DMSO). All stocks were then diluted with complete culture medium to achieve the required concentrations. In all cases, the final concentration of DMSO was 0.4% (v/v) which was found to be nontoxic to the parasite. Compounds were then placed in triplicate wells of 96-well flat-bottom tissue culture grade plates at concentrations ranging between 0 and 100 μM in a final well volume of 100 μL for primary screening. Chloroquine (100 nM) was used as a positive control in all experiments. Parasite culture was synchronized at ring stage with 5% sorbitol [16]. Synchronized culture was aliquoted to drug containing 96-well plates at 2% hematocrit and 1% parasitemia. After 48 h of incubation under standard culture conditions, the plates were harvested and read by the SYBR Green I fluorescence-based method [17] using a 96-well fluorescence plate reader (Victor, Perkin–Elmer), with excitation and emission wavelengths of 497 and 520 nm, respectively. The fluorescence readings were plotted against drug concentrations, and drawing a horizontal line from the 50% value on the growth axis till it intersected with the growth inhibition curve. Then a vertical line was drawn from the point of intersection and projected on the concentration axis to obtain IC_{50} values.

4.3. Measurement of in vitro cytotoxic activity of pyrido[1,2-a] pyrimidin-4-one derivatives against mammalian cell line

Mammalian cell line (HUH-7) was used to determine pyrido[1,2-*a*]pyrimidin-4-one derivatives toxicity by using MTT assay for cell

viability as described by Mosmann [18]. HUH-7 cells were cultured in complete DMEM (cDMEM) containing 10% fetal bovine serum, 0.2% sodium bicarbonate, and 50 μg ml^{-1} gentamicin. Briefly, cells (10^4 cells/200 μl well) were seeded into 96-well flat-bottom tissue-culture plates in complete culture medium. Compounds (ranging between 0 and 200 μM) were added after overnight seeding and incubated for 24 h in a humidified atmosphere at 37 °C and 5% CO_2. DMSO (final concentration 10%) was added as positive control. An aliquot of a stock solution of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) (5 mg mL^{-1} in 1× phosphate-buffered saline) was added at 20 μl per well and incubated for another 3 h. After the plate was spun at 1500 rpm for 5 min, the supernatant was removed and 100 μl of the stop agent DMSO was added to the well to dissolve the formazan crystals. Formation of formazan, an index of growth, was read at 570 nm by 96-well plate reader (Versa Max), and IC_{50} values were determined by analysis of dose–response curves as indicated earlier. The therapeutic index was calculated as IC_{50} (mammalian cell)/IC_{50} (*Pf* 3D7).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.04.031.

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