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Pyrido[1,2-*a*]pyrimidin-4-ones as antiplasmodial falcipain-2 inhibitors

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ABSTRACT

Plasmodium falciparum cysteine protease falcipain-2 (FP-2) is a promising target for antimalarial chemotherapy and inhibition of this protease affects the growth of parasite adversely. A series of pyrido[1,2-*a*]pyrimidin-4-ones were synthesized and evaluated for their in vitro FP-2 inhibitory potential. Compounds (**14**,**17**) showed excellent FP-2 inhibition and can serve as lead compounds for further development of potent FP-2 inhibitors as potential antimalarial drugs.

1. Introduction

Parasite born diseases were having extirpating impact on human civilizations. However, none of the parasitic diseases has had as an enormous impact on humans as that of malaria, which is one of the major causes of morbidity and mortality of human civilizations in the past, and even today, it remains one of the deadliest diseases on the planet.[1] Malaria, particularly the one caused by *Plasmodium falciparum*, remains a serious health problem in Africa, South America and many parts of Asia.[2] 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.[3]

An estimated 600 million people are at risk of contracting malaria infection and the disease kills more than 1 million children each year as well as a large number of pregnant women. About 90% of these casualties occur in tropical Africa and the great majorities are children under the age of five. Exacerbated by poverty, malaria exerts a high toll on populations that can least afford a cure.[4] Despite the toll of tropical parasitic diseases on human life in the developing world, present therapies still rely on drugs that were developed decades ago. The *P. falciparum* genome sequencing has revealed a number of new targets for drug and vaccine development. Development of newer antimalarial drugs only remains an economically and environmentally viable alternative to fight out the menace of the disease.[5]

Hemoglobin degradation is essential to the survival of the parasite, as interruption of this process with a variety of protease inhibitors leads to parasite's death.[6] The hemoglobin degradation pathway involving several parasite specific enzymes like plasmepsins and falcipains plays an important role in providing food to the parasite.[7] One possibility to strike against the parasite is to inhibit an enzyme within this pathway, interrupting the nutrition source and therefore eliminating the parasite by starvation.[8] Erythrocytic malaria parasite degrades hemoglobin as the principal source of amino acids for parasite protein synthesis. One of the potential targets for antimalarial chemotherapy includes an enzyme involved in the processing of hemoglobin that is, cysteine protease enzyme FP-2.[9]

Very few peptides and peptidomimetic compounds have been reported to exhibit FP-2 inhibitory activity in the literature other than some chalcones and semicarbazones. 2-(3,4-Dihydro-4-oxothieno[2,3-*d*]pyrimidin-2-ylthio)acetamides[10] and dihydroartemisinin based thiosemicarbazones[11] were reported as FP-2 inhibitors. Some 4*H*-pyrido[1,2-*a*]pyrimidin-4-ones[12] have been reported earlier in the patent literature for antimalarial and anti-inflammatory activity and 2-chloro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one has been used as a synthone for the synthesis of some new polycyclic heteroaromatic compounds for antimalarial activity.[13]

Herein we report some pyrido[1,2-*a*]pyrimidin-4-ones as potential FP-2 inhibiting antimalarials. It has been tried to incorporate the pharmacophoric features of α,β-unsaturated system of chalcones[14] and of semicarbazones/thiosemicarbazones[15] as urea/amide/carbamates in the designed compounds. The compounds so designed have been synthesized and evaluated for inhibition of FP-2 enzyme (see Fig. 1).

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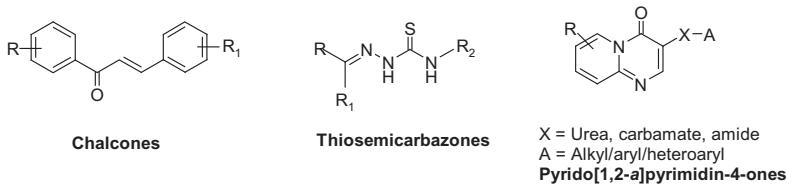


Figure 1. FP-2 inhibiting scaffolds

2. Results and discussion

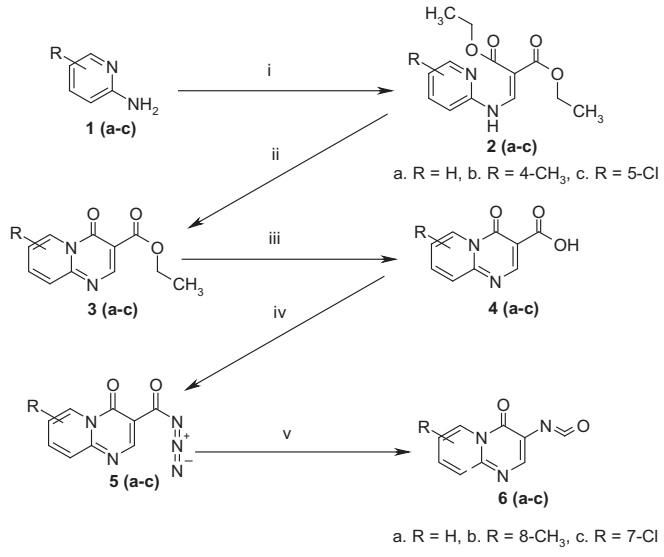
2.1. Chemistry

The targeted compounds were prepared by adopting synthetic strategies outlined in Schemes 1–3. As depicted in Scheme 1 substituted 2-aminopyridines (**1a–c**) by Gould and Jacob coupling reaction with diethyl ethoxymethylenemalonate yielded (**2a–c**), which on cyclization by refluxing in diphenyl ether resulted into pyrido[1,2-*a*]pyrimidin-4-one monoesters (**3a–c**). The esters (**3a–c**) on acid catalyzed hydrolysis offered free acids (**4a–c**). The acids (**4a–c**) when reacted with ethylchloroformate in presence of triethylamine yielded anhydrides which on further in situ reaction with sodium azide yielded the azides (**5a–c**). The azides (**5a–c**) offered isocyanate derivatives (**6a–c**) (Scheme 1) by refluxing them in toluene.

The ureas (**7–26**) (Scheme 2) were prepared by coupling of suitable isocyanate **(6a–c)** with amine derivatives in toluene under reflux conditions following the procedure as depicted in Scheme 2. The carbamates (**27–29**) were synthesized by coupling of suitable isocyanate **(6a–c)** with aryl alcohols in toluene under reflux conditions. The amides (**30–32**) (Scheme 3) were prepared by EDC coupling of a suitable acid (**4a–c**) with amine derivatives.

2.2. Biology

Compounds (**7–32**) were evaluated for their inhibitory activity against recombinant FP-2 using Cbz-Phe-Arg-AMC as fluorogenic substrate.[9] Preliminary screening was performed at 10 μM concentration. An equivalent concentration of DMSO was used as negative control and the irreversible standard inhibitor of clan CA family C1 cysteine proteases (papain family), namely E-64 was used as positive control. Assays were performed to determine the percentage inhibition of the enzyme at a concentration of 10 μM. FP-2 activity is assessed by cleavage of the fluorogenic substrate Cbz-Phe-Arg-AMC releasing the fluorescent AMC group. Hence decrease in fluorescence intensity in a sample represents inhibition of enzyme activity. The IC_{50} values were determined for those compounds only which showed more than 40% inhibition at 10 μM concentration.



Scheme 1. Reagents and conditions: (i) EMME, reflux (ii) DPE, reflux (iii) HCl, reflux (iv) (a) ECF, TEA, DMF, 0 °C (b). NaN_3, H_2O, 0 °C (v) toluene, reflux.

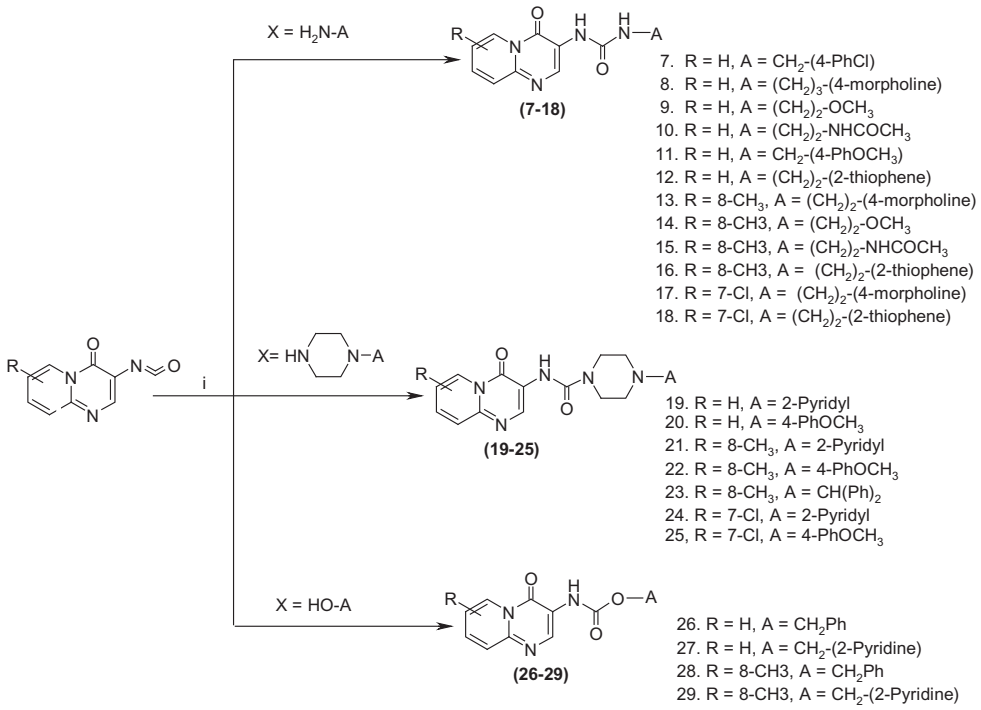
The enzyme inhibition data given in Table 1 shows that the pyrido[1,2-*a*]pyrimidin-4-ones show FP-2 inhibition in micromolar range. Based on these results a broad structure–activity relationship could be deduced for this series of compounds. Urea moiety has the potential to provide potent FP-2 inhibitors as the carbamate and carboxamide derivatives are much less active in comparison to the disubstituted ureas.

Urea derivatives of ethylmorpholino (**17**, IC_{50} 7 μM) and methoxyethyl (**14**, IC_{50} 6 μM) proved to be the most potent compounds among the whole lot. 2-Thiophenethyl (**12**, IC_{50} 14 μM; **16**, IC_{50} 21 μM; **18**, IC_{50} 23 μM), acetamidoethyl (**10**, IC_{50} 16 μM) and 4-methoxyphenylpiperazine (**22**, IC_{50} 19 μM) were the other groups which gave active compounds when attached to one end of urea moiety. 2-Pyridylpiperazine is another moiety which offered quite active compounds (**24**, IC_{50} 12 μM; **19**, IC_{50} 15 μM).

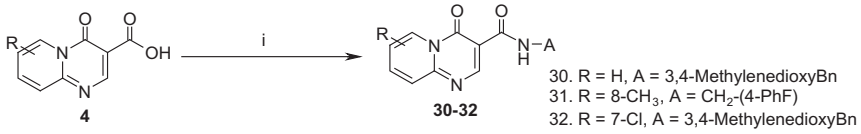
In general 8-methyl and 7-chloro substituted derivatives offered more potent compounds indicating that increasing the steric size of the group attached to the B-ring (i.e., pyrido) is likely to provide more potent FP-2 inhibitors. An electron rich environment at the end of the carbon chain attached to the urea nitrogen also seems to be conducive for better activity as indicated by the compounds having morpholino, 2-thiophenenyl, 2-pyridyl, 4-methoxyphenyl, acetamidoethyl and 2-methoxyethyl groupings. But bulky (**23**, benzhydrylpiperazine) and (**8**, 4-morpholinopropyl) groupings may not offer potent derivatives.

3. Conclusion

There is a need for development of new drug molecules for the control of malaria parasite as the parasite is developing resistance to the older drugs. Inhibition of FP-2 could be an efficient strategy to kill the parasite by starvation due to the blockade of catabolic chain, essential for obtaining growth nutrients. In this regards we planned to develop parasitic-enzyme-specific inhibitors. For identification of FP-2 inhibitors, we have synthesized thirty two pyrido[1,2-*a*]pyrimidin-4-one derivatives, which showed good FP-2 inhibitory potential. The compounds like (**10**, IC_{50} 16 μM), (**12**, IC_{50} 14 μM), (**14**, IC_{50} 6 μM), (**16**, IC_{50} 21 μM), (**17**, IC_{50} 7 μM), (**18**, IC_{50} 23 μM), (**19**, IC_{50} 15 μM), (**22**, IC_{50} 19 μM), (**24**, IC_{50} 12 μM) and (**29**, IC_{50} 18 μM) are showing excellent FP-2 inhibition. Based on the activity data of the synthesized compounds, pyrido[1,2-*a*]pyrimidin-4-one can serve as a lead series for future investigations. By appropriate structural optimizations the potency of this series of compounds can be improved substantially against FP-2 enzyme which in turn can serve to be potential antimalarial agents.



Scheme 2. Reagents and conditions: (i) X, toluene, reflux.



Scheme 3. Reagents and conditions:(i) X, EDC, HOBT, DIEA, MDC, 0 °C, RT.

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 blok 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 ^{1}H NMR spectra (on a Bruker 400 MHz spectrometer) were recorded in DMSO-d_6 (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 Perkin-Elmer/Carlo-Erba elemental analyzer.

4.1.1. Diethyl 2-pyridylaminomethylenemalonate (2a)

A mixture of 2-aminopyridine (**1a**) (10 g, 10.6 mmol) and diethyl ethoxymethylenemalonate (24.47 g, 10.6 mmol) was refluxed for 2 h. The reaction mixture was cooled to RT to get (**2a**) as a light brown solid (14 g, 50%), mp 68–69 °C (Lit.[16,17] 67.5–68 °C). R_f 0.85 (EtOAc). IR (KBr): 3274, 1686, 1649, 1601, 1248 cm^{-1}. ^{1}H NMR: 1.22–1.29 (m, 6H, CH_3), 4.12–4.18 (q, 2H, CH_2), 4.20–4.25 (q, 2H, CH_2), 7.14–7.17 (m, 1H, Ar-H), 7.39–7.41 (d, 1H, Ar-H), 7.79–7.83 (m, 1H, Ar-H), 8.36–8.37 (d, 1H, Ar-H), 9.05–9.08 (d, 1H, –NH–C*H*═C), 10.79–10.82 (d, 1H, N–*H*). MS: (*m*/*z*) 265 (M^++1).

4.1.2. Ethyl 4-oxo-4*H*-pyrido[1,2-*a*]pyrimidine-3-carboxylate (3a)

The pyridine derivative (**2a**) (13 g, 5.9 mmol) was refluxed in diphenyl ether (100 ml) using air condenser for 8 h in a two-neck round-bottom flask (250 ml). The reaction mixture was cooled to RT and added hexane (500 ml) in to it to get a solid precipitate. The precipitated solid was filtered, washed with hexane and dried to obtain compound (**3a**) (10 g, 93.1%), mp 111–13 °C (Lit.[16] 110–11 °C). R_f 0.48 (EtOAc). IR (KBr): 1730, 1482, 1288, 1227 and 1104 cm^{-1}. MS: (*m*/*z*) 219 (M^++1).

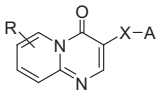
4.1.3. 4-Oxo-4*H*-pyrido[1,2-*a*]pyrimidine-3-carboxylic acid (4a)

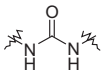
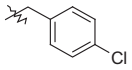
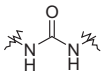
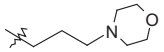
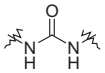
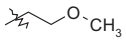
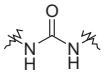
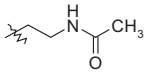
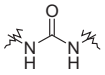
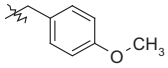
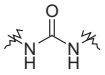
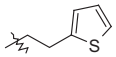
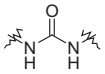
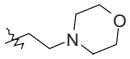
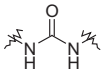
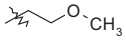
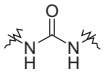
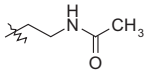
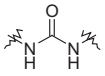
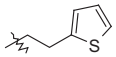
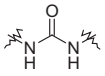
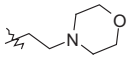
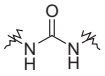
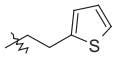
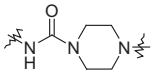
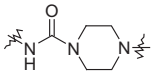
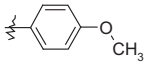
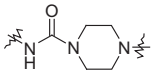
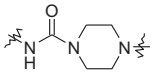
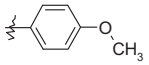
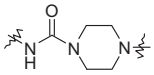
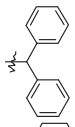
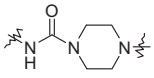
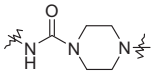
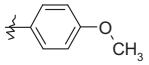
A solution of the ester (**3a**) (10 g, 5.2 mmol) in conc. HCl (15 ml) was refluxed for 6 h. The reaction mixture was cooled to RT to get a solid precipitate. The solid precipitate was filtered, washed with ether and dried to get compound (**4a**) as a dark brown solid (8 g, 85.5%), mp. 268 °C (decomp.) [Lit.[16] 265 °C (decomp.)]. R_f 0.1 (EtOAc). IR (KBr): 3091, 1757, 1677, 1636, 1519, 1483, 1275, 1247 cm^{-1}. ^{1}H NMR: 7.67–7.71 (m, 1H, Ar-H), 7.97–8.00 (d, 1H, Ar-H), 8.31–8.35 (m, 1H, Ar-H), 8.96 (s, 1H, Ar-H), 9.22–9.23 (d, 1H, Ar-H). MS: (*m*/*z*) 191 (M^++1).

4.1.4. 4-Oxo-4*H*-pyrido[1,2-*a*]pyrimidine-3-carbonylazide (5a)

In a two-neck round-bottom flask (250 ml), compound (**4a**) (8 g, 4.2 mmol) and triethylamine (8.5 g, 8.4 mmol) were dissolved in

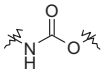
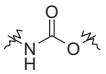
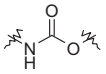
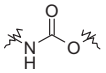
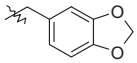
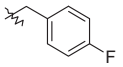
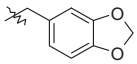
Table 1
Inhibition of FP-2 in percentage and IC_{50} compounds of 7–32



Sr. No.	R	X	A	Falcipain-2 inhibition (%)	IC_{50} (μM)
7	H			39	nd
8	H			25	nd
9	H			31	nd
10	H			46	16
11	H			18	nd
12	H			44	14
13	8-CH_3			27	nd
14	8-CH_3			63	6
15	8-CH_3			15	nd
16	8-CH_3			45	21
17	7-Cl			68	7
18	7-Cl			42	23
19	H			46	15
20	H			43	nd
21	8-CH_3			37	nd
22	8-CH_3			46	19
23	8-CH_3			23	nd
24	7-Cl			41	12
25	7-Cl			36	nd

(*continued on next page*)

Table 1 (*continued*)

Sr. No.	R	X	A	Falcipain-2 inhibition (%)	IC_{50} (μM)
26	H			12	nd
27	H			18	nd
28	8-CH_3			31	nd
29	8-CH_3			42	18
30	H			7	nd
31	8-CH_3			6	nd
32	7-Cl			24	nd
33	E-64	—	—	92(2μM)	0.02
34	DMSO	—	—	0	—

nd = not determined

DMF (25 ml). The reaction mixture was cooled to 0 °C. Ethyl chloroformate (5.03 g, 4.6 mmol) was added to the reaction mixture and stirred for 15 min followed by addition of aqueous solution of sodium azide (10.95 g, 16.8 mmol) resulting into a yellow solid precipitate. The solid precipitate was filtered, washed with cold water; hexane and then air dried affording the compound (**5a**) as brown solid (6.8 g, 75.1%) mp 130–31 °C. R_f 0.28 (EtOAc) IR (KBr): 2310, 1725, 1472, 1185, 1012 and 787 cm^{-1}. ^1H NMR: 7.61–7.64 (m, 1H, Ar-H), 7.87–7.91 (d, 1H, Ar-H), 8.24–8.35 (m, 1H, Ar-H), 8.94 (s, 1H, Ar-H), 9.10–9.28 (d, 1H, Ar-H). MS: (*m*/*z*) 214 (M^+−1).

4.1.5. 3-Isocyanato-4*H*-pyrido[1,2-*a*]pyrimidin-4-one (6a)

The acid azide (**5a**) (4 g, 1.8 mmol) in toluene was refluxed for 2 h. Work up of the reaction mixture was done by removing toluene under vacuum to get a light brown solid compound (**6a**) (3.2 g, 40.6%), mp 130–31 °C. R_f 0.5 (EtOAc). IR (KBr): 2300, 1687, 1650, 1575, 1550 1490 and 1190 cm^{-1}. MS: (*m*/*z*) 188 (M^++1).

4.1.6. Diethyl(4-methyl-2-pyridylamino)methylene-malonate (2b)

4-Methyl-2-aminopyridine (10 g, 9.25 mmol) was reacted with diethyl ethoxymethylenemalonate (21.29 g, 9.25 mmol) as described above for compound (**2a**), affording (**2b**) (15 g, 58.2%), mp 73–74 °C (Lit.[16] 72–73 °C). R_f 0.9 (EtOAc). IR (KBr): 1731, 1682, 1648, 1605, 1548, 1374 and 1218 cm^{-1}. MS: (*m*/*z*) 279 (M^++1).

4.1.7. Ethyl 8-methyl-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidine-3-carboxylate (3b)

In analogy to compound (**3a**), (**2b**) (10 g, 4.31 mmol) was refluxed in diphenylether to obtain (**3b**) (10 g, 88.4%), mp 174–76 °C (Lit.[16] 171–72 °C). R_f 0.55 (EtOAc). IR (KBr): 1689, 1639, 1556, 1501, 1284 and 1143 cm^{-1}. ^1H NMR: 1.28–1.32 (t, 3H, *J* = 7.2 Hz, CH_3), 2.51 (s, 3H, CH_3), 4.23–4.29 (q, 2H, *J* = 7.2 Hz, CH_2), 7.42–7.44 (d, 1H, *J* = 7.2 Hz, Ar-H), 7.67 (s, 1H, Ar-H), 8.82 (s, 1H, Ar-H), 9.04–9.05 (d, 1H, *J* = 7.2 Hz, Ar-H). MS: (*m*/*z*) 233 (M^++1).

4.1.8. 8-Methyl-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidine-3-carboxylic acid (4b)

A solution of the ester (**3b**) (10 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 get 8-methyl-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-carboxylic acid (**4b**) (8.5 g, 96.7%), mp 225 °C [Lit.[18] 223 °C]. R_f 0.11(EtOAc). IR (KBr): 2645, 1764, 1696, 1620, 1522, 1393, 1268, 1204 and 1152 cm^{-1}. ^1H NMR: 2.62 (s, 3H, CH_3), 7.62–7.64 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.89 (s, 1H, Ar-H), 8.90 (s, 1H, Ar-H), 9.14–9.16 (d, 1H, *J* = 8.0 Hz, Ar-H), 10.9 (b, 1H, COO*H*). MS: (*m*/*z*) 205 (M^++1).

4.1.9. 8-Methyl-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidine-3-carbonylazide (5b)

Compound (**5b**) was prepared by reacting the acid (**4b**) (8 g, 3.9 mmol) with ethyl chloroformate (4.68 g, 4.3 mmol) and sodium azide (10.19 g, 15.7 mmol) as described above for compound (**5a**) yielding the compound (**5b**) (7 g, 77.9%), mp 148–50 °C. R_f 0.35 (EtOAc). IR (KBr): 2142, 1712, 1640, 1569, 1485, 1285 and 1172 cm^{-1}. ^1H NMR: 2.58 (s, 3H, CH_3), 7.49–7.51 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.75 (s, 1H, Ar-H), 8.83 (s, 1H, Ar-H), 9.09–9.11 (d, 1H, *J* = 8.0 Hz, Ar-H). MS: (*m*/*z*) 230 (M^++1).

4.1.10. 3-Isocyanato-8-methyl-4*H*-pyrido[1,2-*a*]pyrimidin-4-one (6b)

The azide (**5b**) (4 g) was refluxed in toluene for 2 h. The reaction mixture was cooled to RT and the solvent was removed under *vacuo* to get 3-isocyanato-8-methyl-4*H*-pyrido[1,2-*a*]pyrimidin-4-one (**6b**) (3.34 g, 95.1%), mp 234–35 °C. R_f 0.61 (EtOAc). IR (KBr): 2223, 1670, 1639, 1482, 811 and 764 cm^{-1}. MS: (*m*/*z*) 202 (M^++1).

4.1.11. Diethyl (5-chloro-2-pyridylamino)methylene malonate (2c)

Compound (**2c**) was prepared by reacting the compound (**1c**) (10 g, 7.8 mmol) with diethyl ethoxymethylenemalonate (17.96 g, 7.8 mmol) as described above for compound (**2a**), affording the compound (**2c**) (15 g, 42.9%), mp 132–34 °C (Lit.[19] 131–32 °C). R_f 0.91 (EtOAc). IR (KBr): 3270, 1730, 1676, 1644, 1588, 1552, 1465,

1395, 1219 and 1007 cm^{-1}. ^1H NMR: 1.22–1.29 (m, 6H, CH_3), 4.13–4.26 (m, 4H, CH_2), 7.47–7.49 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.91–7.93 (d, 1H, *J* = 8.0 Hz, Ar-H), 8.41 (s, 1H, Ar-H), 8.94–8.97 (d, 1H, *J* = 12.0 Hz, –NHC*H*=C), 10.79–10.82 (d, 1H, *J* = 12.0 Hz, N–*H*). MS: (*m/z*) 299 (M^++1).

4.1.12. Ethyl 7-chloro-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidine-3-carboxylate (3c)

With a procedure similar to that adopted for compound (**3a**) compound (**3c**) was prepared from compound (**2c**) (15 g, 5.03 mmol) affording the titled compound (**3c**) (10 g, 78.8%), mp 146–47 °C (Lit.[19] 147–48 °C). R_f 0.54 (EtOAc). IR (KBr): 1681, 1645, 1591, 1552, 1466, 1366, 1222 and 1091 cm^{-1}. MS: (*m/z*) 253 (M^++1).

4.1.13. 7-Chloro-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidine-3-carboxylic acid (4c)

In analogy to compound (**4a**), (**4c**) was obtained by employing the same procedure using (**3c**) (10 g, 3.9 mmol) to get 7-chloro-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidine-3-carboxylic acid (**4c**) (8 g, 89.9%), mp 256–57 °C. R_f 0.12 (EtOAc). IR (KBr): 3063, 1759, 1661, 1612, 1510, 1418, 1263 and 1163 cm^{-1}. MS: (*m/z*) 225 (M^++1).

4.1.14. 7-Chloro-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidine-3-carbonylazide (5c)

Compound (**5c**) was prepared by reacting compound (**4c**) (8 g, 6.25 mmol) with ethyl chloroformate (6.78 g, 6.87 mmol) and sodium azide (16.25 g, 25 mmol) as described above for compound (**5a**) affording the compound (**5c**) (6.5 g, 74.2%), mp 158–60 °C. R_f 0.25 (EtOAc). IR (KBr): 2158, 1732, 1563, 1472, 1296, 1201 and 1023 cm^{-1}. MS: (*m/z*) 250 (M^++1).

4.1.15. 7-Chloro-3-isocyanato-4*H*-pyrido[1,2-*a*]pyrimidin-4-one (6c)

(**5c**) (4 g, 1.8 mmol) was refluxed in toluene for 2 h. The reaction mixture was concentrated to get 7-chloro-3-isocyanato-4*H*-pyrido[1,2-*a*]pyrimidin-4-one (**6c**) (3.34 g, 98.5%), mp 312–13 °C. R_f 0.65 (EtOAc). IR (KBr): 2243, 1668, 1625, 1547, 1525, 1475 and 1182 cm^{-1}. MS: (*m/z*) 222 (M^++1).

4.1.16. 1-(4-Chlorobenzyl)-3-(4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)urea (7)

The isocyanate (**6a**) (0.5 g, 0.27 mmole) and 4-chlorobenzylamine (0.227 g, 0.16 mmol) in toluene (25 ml) were refluxed together for 6 h. The reaction mixture was cooled to RT to get a solid precipitate. The solid precipitate was filtered and washed with hexane. The product was purified by column chromatography using ethyl acetate (80%) in hexane as eluant to get the titled compound (**7**) (0.2 g, 37.9%), mp 250–51 °C. R_f 0.6 (EtOAc). IR (KBr): 3318, 1693, 1637, 1536, 1479, 1220 and 1136 cm^{-1}. ^1H NMR: 4.39–4.41 (d, 2H, *J* = 6.0 Hz, CH_2), 7.26–7.35 (m, 3H, N–*H* & Ar-H), 7.41–7.56 (m, 3H, Ar-H), 7.61–7.64 (d, 1H, *J* = 9.2 Hz, Ar-H), 7.68–7.71 (m, 1H, Ar-H), 8.55 (s, 1H, N–*H*), 8.85–8.87 (d, 1H, *J* = 7.2 Hz, Ar-H), 9.16 (s, 1H, Ar-H). MS: (*m/z*) 328.9 (M^+).

4.1.17. 1-[3-(Morpholin-4-yl)propyl]-3-(4-oxo-4*H*-pyrido [1,2-*a*]pyrimidin-3-yl)urea (8)

A mixture of (**6a**) (0.5 g, 0.27 mmol), 3-(4-morpholino)propylamine (0.227 g, 0.16 mmol) and toluene (25 ml) was refluxed for 6 h to get a solid precipitate. The solid precipitate was filtered and washed with hexane. The crude product was purified by column chromatography using ethyl acetate (90%) in hexane as eluant to obtain the titled compound (**8**) (0.18 g, 40.6%), mp 188–90 °C. R_f 0.67 (EtOAc). IR (KBr): 3297, 1663, 1549, 1479, 1228 and 1118 cm^{-1}. ^1H NMR: 1.55–1.62 (m, 2H, CH_2), 2.28–2.34 (m, 6H, NCH_2), 3.10–3.15 (m, 2H, CH_2), 3.56–3.58 (t, 4H, *J* = 4.6 Hz, OCH_2), 7.01–7.04 (t, 1H, *J* = 7.4 Hz, N–*H*), 7.23–7.27 (m, 1H, Ar-H), 7.60–7.63 (d, 1H, *J* = 8.8 Hz, Ar-H), 7.67–7.71 (m, 1H, Ar-H), 8.29 (s, 1H, N–*H*), 8.84–8.86 (d, 1H, *J* = 6.8 Hz, Ar-H), 9.17 (s, 1H, Ar-H). MS: (*m/z*) 332 (M^++1).

4.1.18. 1-(2-Methoxyethyl)-3-(4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)urea (9)

Compound (**9**) was prepared by reacting the isocyanate (**6a**) (0.3 g, 0.16 mmol) with 2-methoxyethylamine (0.119 g, 0.16 mmol) as previously described for compound (7). Chromatographic purification of the crude product using methanol (0.9%) in ethyl acetate as eluant yielded the compound (**9**) (0.5 g, 61.1%), mp 195–97 °C. R_f 0.65 (1% MeOH in EtOAc). IR (KBr): 3362, 1696, 1639, 1548, 1463, 1226, 1192 and 1099 cm^{-1}. ^1H NMR: 3.25–3.28 (m, 5H, NCH_2 & OCH_3), 3.38–3.39 (t, 2H, *J* = 5.2 Hz, OCH_2), 7.15–7.18 (t, 1H, *J* = 5.4 Hz, N–*H*), 7.23–7.27 (m, 1H, Ar-H), 7.60–7.63 (d, 1H, *J* = 9.2 Hz, Ar-H), 7.67–7.72 (m, 1H, Ar-H), 8.41 (s, 1H, N–*H*), 8.84–8.86 (d, 1H, *J* = 6.8 Hz, Ar-H), 9.16(s, 1H, Ar-H). MS: (*m/z*) 263 (M^++1).

4.1.19. *N*-{2-[3-(4-Oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl) ureido]ethyl}acetamide (10)

In analogy to compound (7), (**10**) was obtained by refluxing the isocyanate (**6a**) (0.3 g, 0.16 mmol) with 2-acetamidoethylamine (0.62 g, 0.16 mmol). Chromatographic purification of the crude product using methanol (0.9%) in ethyl acetate as eluant offered compound (**10**) (0.5 g, 61.1%), mp 247–49 °C. R_f 0.45 (5% MeOH in EtOAc). IR (KBr): 3317, 3251, 1726, 1633, 1536, 1467, 1219 and 1120 cm^{-1}. ^1H NMR: 1.81 (s, 3H, CH_3), 3.11–3.16 (m, 4H, NCH_2), 7.09–7.11 (t, 1H, *J* = 5.0 Hz, N–*H*), 7.23–7.27 (m, 1H, Ar-H), 7.61–7.63 (d, 1H, *J* = 8.8 Hz, Ar-H), 7.68–7.72 (m, 1H, Ar-H), 7.94 (b, 1H, N–*H*), 8.35 (s, 1H, N–*H*), 8.84–8.86 (d, 1H, *J* = 7.2 Hz, Ar-H), 9.17 (s, 1H, Ar-H). MS: (*m/z*) 290 (M^+-1).

4.1.20. 1-(4-Methoxybenzyl)-3-(4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)urea (11)

Compound (**11**) was prepared by treating the isocyante (**6a**) (0.3 g, 0.16 mmol) with 4-methoxybenzylamine (0.172 g, 0.16 mmol) as described above for compound (7). The crude product was purified by column chromatography using methanol (1%) in ethyl acetate as eluant offering the compound (**11**) (0.15 g, 51.9%), mp 228–30 °C. R_f 0.68 (1% MeOH in EtOAc). IR (KBr): 3361, 3277, 1639, 1531, 1242, 1220, 1180 and 1122 cm^{-1}. ^1H NMR: 3.73 (s, 3H, OCH_3), 4.24–4.25 (d, 2H, *J* = 6 Hz, CH_2), 6.89–6.91 (d, 2H, *J* = 8.8 Hz, Ar-H), 7.22–7.27 (m, 3H, N–*H* & Ar-H), 7.40–7.43 (m, 1H, Ar-H), 7.61–7.63 (d, 1H, *J* = 9.2 Hz, Ar-H), 7.68–7.72 (m, 1H, Ar-H), 8.39 (s, 1H, N–*H*), 8.84–8.86 (d, 1H, *J* = 7.2 Hz, Ar-*H*), 9.18 (s, 1H, Ar-H). MS: (*m/z*) 325 (M^++1).

4.1.21. 1-(4-Oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)-3-(2-thiophen-2-ylethyl)urea (12)

A mixture of isocyanate (**6a**) (0.3 g, 0.16 mmol), 2-(2-thiophenyl)ethylamine (0.204 g, 0.16 mmol) and toluene was refluxed for 6 h. The reaction mixture was cooled to RT to get solid precipitate. The solid precipitate so obtained was filtered and the crude product was purified by column chromatography using methanol (2%) in ethyl acetate as eluant to get the compound (**12**) (0.2 g, 39.6%), mp 216–19 °C. R_f 0.5 (1% MeOH in EtOAc). IR (KBr): 3213, 3033, 1656, 1525, 1469, 1218, 1182, 1127, 758 and 695 cm^{-1}. ^1H NMR: 2.97–3.01 (t, 2H, *J* = 6.6 Hz, CH_2), 3.37–3.40 (m, 2H, CH_2), 6.92–6.93 (d, 1H, *J* = 2.4 Hz, Ar-*H*), 6.97–6.99 (m, 1H, Ar-H), 7.15–7.18 (t, 1H, *J* = 5.4 Hz, N–*H*), 7.23–7.27 (m, 1H, Ar-H), 7.35–7.37 (d, 1H, *J* = 5.2 Hz, Ar-H), 7.61–7.63 (d, 1H, *J* = 8.8 Hz, Ar-H), 7.68–7.72 (m, 1H, Ar-H), 8.41 (s, 1H, N–*H*), 8.84–8.85 (d, 1H, *J* = 7.2 Hz, Ar-H), 9.19 (s, 1H, Ar-H). MS: (*m/z*) 315 (M^++1).

4.1.22. 1-(8-Methyl-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)-3-[2-(morpholin-4-yl)ethyl]urea (13)

The isocyanate derivative (**6b**) (0.3 g, 0.149 mmol) was reacted with 2-(4-morpholino)ethylamine (0.163 g, 0.149 mmol) as described above for compound (**7**). Chromatographic purification of the crude product using methanol (0.5%) in ethyl acetate as eluant offered the compound (**13**) (0.21 g, 42.5%), mp 225–26 °C. R_f 0.64 (1% MeOH in EtOAc). IR (KBr): 3334, 1632, 1569, 1460, 1385, 1235, 1104 and 1002 cm^{-1}. ^1H NMR: 2.37–2.41 (m, 9H, CH_3 & NCH_2), 3.21–3.24 (m, 2H, CH_2), 3.58–3.59 (m, 4H, CH_2), 6.99 (b, 1H, N–*H*), 7.11–7.12 (d, 1H, *J* = 7.2 Hz, Ar-H), 7.42 (s, 1H, Ar-H), 8.37 (s, 1H, N–*H*), 8.76–8.78 (d, 1H, *J* = 7.2 Hz, Ar-H), 9.09 (s, 1H, Ar-H) MS: (*m/z*) 332 (M^++1).

4.1.23. 1-(2-Methoxyethyl)-3-(8-methyl-4-oxo-4H-pyrido [1,2-a]pyrimidin-3-yl)urea (14)

Compound (**14**) was prepared by reacting compound (**6b**) (0.3 g, 0.149 mmol) with 2-methoxyethylamine (0.111 g, 0.149 mmol) as described above for compound (**7**). Work up of the reaction mixture followed by chromatographic purification using ethyl acetate (95%) in hexane as eluant afforded compound (**14**) (0.16 g, 38.84%). mp 232–34 °C. R_f 0.75 (1% MeOH in EtOAc). IR (KBr): 3388, 3305, 1633, 1534, 1452, 1228 cm^{-1}. ^1H NMR: 2.39 (s, 3H, CH_3), 3.22–3.25 (m, 2H, CH_2), 3.26 (s, 3H, OCH_3), 3.35 (m, 2H, CH_2), 7.09–7.11 (m, 1H, N–*H*), 7.41 (s, 1H, Ar-H), 8.30 (s, 1H, N–*H*), 8.73–8.75 (d, 1H, *J* = 7.6 Hz, Ar-H), 9.06 (s, 1H, Ar-H). MS: (*m/z*) 276 (M^+−1).

4.1.24. *N*-{2-[3-(8-Methyl-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin- 3-yl)ureido]- ethyl}acetamide (15)

Compound (**15**) was prepared by reacting the compound (**6b**) (0.3 g, 0.149 mmol) with 2-acetamidoethylamine (0.151 g, 0.149 mmol) as described above for compound (**7**). Chromatographic purification of the crude product was done using ethyl acetate (95%) in hexane as eluant to get the desired product (**15**) (0.2 g, 44.2%), mp 269–70 °C. R_f 0.7 (1% MeOH in EtOAc). IR (KBr): 3356, 3289, 1633, 1524, 1451, 1239 and 1004 cm^{-1}. ^1H NMR: 1.81 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 3.11–3.15 (m, 4H, CH_2), 7.04–7.06 (t, 1H, *J* = 5.0 Hz, N–*H*) 7.11–7.13 (d, 1H, *J* = 7.2 Hz, Ar-H), 7.43 (s, 1H, Ar-H), 7.93 (b, 1H, N–*H*), 8.26 (s, 1H, N–*H*), 8.76–8.78 (d, 1H, *J* = 7.2 Hz, Ar-H), 9.09 (s, 1H, Ar-H). MS: (*m/z*) 304 (M^++1).

4.1.25. 1-(8-Methyl-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)-3-(2-thiophen-2-ylethyl)urea (16)

Compound (**6b**) (0.3 g, 0.149 mmol) was treated with 2-(2-thiophenyl)ethylamine (0.188 g, 0.149 mmol) as described above for compound (**7**). Chromatographic purification of the crude product was done using methanol (0.2%) in ethyl acetate as eluant to obtain 1-(8-methyl-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)-3-(2-thiophen-2-yl-ethyl)urea (**16**) (0.2 g, 40.8%), mp 248–51 °C. R_f 0.78 (1% MeOH in EtOAc). IR (KBr): 3278, 1634, 1518, 1449, 1237 and 684 cm^{-1}. ^1H NMR: 2.41 (s, 3H, CH_3), 2.95–2.98 (t, 2H, *J* = 6.4 Hz, CH_2), 3.55–3.77 (m, 2H, NCH_2), 6.92 (b, 1H, Ar-H), 6.97–6.98 (m, 1H, Ar-H), 7.11–7.12 (m, 1H, NH & Ar-H), 7.35–7.37 (d, 1H, *J* = 4.8 Hz, Ar-H), 7.43 (s, 1H, Ar-H), 8.32 (s, 1H, N–*H*), 8.75–8.77 (d, 1H, *J* = 7.2 Hz, Ar-H), 9.11 (s, 1H, Ar-H). MS: (*m/z*) 329 (M^++1).

4.1.26. 1-(7-Chloro-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)-3-[2-(morpholin-4-yl)ethyl]urea (17)

With a procedure similar to that adopted for compound (**7**), (**17**) was prepared by reacting the compound (**6c**) (0.3 g, 0.135 mmol) with 2-(4-morpholino)ethylamine (0.176 g, 0.135 mmol). Chromatographic purification of the crude product using methanol (2%) in ethyl acetate as eluant yielded the titled compound (**17**) (0.22 g, 46.2%), mp 226–27 °C. R_f 0.5 (2% MeOH in EtOAc). IR (KBr): 3285, 1729, 1646, 1552, 1464, 1284, 1247 and 1117 cm^{-1}. ^1H NMR: 2.56 (b, 6H, NCH_2), 3.24–3.27 (m, 2H, CH_2), 3.60 (b, 4H, OCH_2), 7.08 (b, 1H, N–*H*), 7.63–7.65 (d, 1H, *J* = 9.6 Hz, Ar-H), 7.70–7.73 (d, 1H, *J* = 9.6 Hz, Ar-H), 8.57 (s, 1H, N–*H*), 8.84 (s, 1H, Ar-H), 9.17 (s, 1H, Ar-H). MS: (*m/z*) 352 (M^++1).

4.1.27. 1-(7-Chloro-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)-3-(2-thiophen-2-ylethyl)urea (18)

Compound (**18**) was obtained by treating the compound (**6c**) (0.3 g, 0.135 mmol) with 2-(2-thiophenyl)ethylamine (0.17 g, 0.135 mmole) as described above for compound (**7**). The chromatographic purification of the crude product using ethyl acetate (90%) in hexane as eluant yielded the titled compound (**18**) (0.24 g, 50.8%), mp 215–17 °C. R_f 0.75 (1% MeOH in EtOAc). IR (KBr): 3387, 1651, 1544, 1510, 1470, 1224 and 1189 cm^{-1}. ^1H NMR: 2.97–3.00 (m, 2H, CH_2), 3.37–3.40 (m, 2H, CH_2), 6.92–6.93 (d, 1H, *J* = 2.8 Hz, Ar-H), 6.97–6.99 (m, 1H, Ar-*H*), 7.18–7.20 (t, 1H, *J* = 5.6 Hz, N–*H*), 7.35–7.37 (d, 1H, *J* = 5.2 Hz, Ar-H), 7.63–7.66 (d, 1H, *J* = 9.6 Hz, Ar-H), 7.71–7.74 (dd, 1H, *J* = 2.6 Hz and *J* = 9.6 Hz, Ar-H), 8.51 (s, 1H, N–*H*), 8.83–8.84 (d, 1H, *J* = 2.6 Hz, Ar-H), 9.19 (s, 1H, Ar-H). MS: (*m/z*) 349 (M^+).

4.1.28. *N*-(4-Oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)-4-(pyridin-2-yl)piperazine-1-carboxamide (19)

Compound (**19**) was prepared by reacting the compound (**6a**) (0.3 g, 0.16 mmole) with 4-(2-pyridyl)piperazine (0.262 g, 0.16 mmol) as described above for compound (**7**). The crude product was purified by column chromatography using ethyl acetate (90%) in hexane as eluant to afford compound (**19**) (0.19 g, 33.8%), mp 192–95 °C. R_f 0.51 (1% MeOH in EtOAc). IR (KBr): 3393, 1659, 1596, 1537, 1482, 1238 and 762 cm^{-1}. ^1H NMR: 3.58 (b, 8H, NCH_2), 6.65–6.69 (m, 1H, Ar-H), 6.86–6.88 (d, 1H, *J* = 8.8 Hz, Ar-H), 7.31–7.35 (m, 1H, Ar-H), 7.54–7.58 (m, 1H, Ar-H), 7.67–7.69 (m, 1H, Ar-H), 7.80–7.84 (m, 1H, Ar-H), 8.08 (s, 1H, N–*H*), 8.13–8.14 (d, 1H, *J* = 3.6 Hz, Ar-H), 8.77 (s, 1H, Ar-H) 8.91–8.93 (d, 1H, *J* = 7.2 Hz, Ar-H). MS: (*m/z*) 351 (M^++1).

4.1.29. *N*-(4-Oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)-4-(4-methoxyphenyl)piperazine-1-carboxamide (20)

The urea derivative (**20**) was obtained by refluxing the isocyanate (**6a**) (0.3 g, 0.16 mmol) with 4-(4-methoxyphenyl)piperazine (0.308 g, 0.16 mmol) in toluene for 6 h. The reaction mixture was cooled to RT and the solid precipitate so obtained was filtered. Chromatographic purification of the crude product using ethyl acetate (90%) in hexane as eluant yielded the titled compound (**20**) (0.19 g 31.2%), mp 143–45 °C. R_f 0.52 (1% MeOH in EtOAc). IR (KBr): 3396, 1649, 1522, 1479, 1437, 1229 and 1021 cm^{-1}. ^1H NMR: 2.99 (b, 4H, CH_2), 3.61(b, 4H, CH_2), 3.81 (s, 3H, OCH_3), 6.87–7.01 (m, 4H, Ar-H), 7.32–7.35 (m, 1H, Ar-H), 7.67–7.69 (d, 1H, *J* = 9.2 Hz, Ar-H), 7.80–7.84 (m, 1H, Ar-H), 8.06 (s, 1H, N–*H*), 8.77 (s, 1H, Ar-H), 8.91–8.93 (d, 1H, *J* = 7.2 Hz, Ar-H). MS: (*m/z*) 380 (M^++1).

4.1.30. *N*-(8-Methyl-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)-4-(pyridin-2-yl)piperazine-1-carboxamide (21)

With a procedure similar to that adopted for compound (**7**), (**6b**) (0.3 g, 0.149 mmol) was reacted with 4-(2-pyridyl)piperazine (0.243 g, 0.149 mmol). Chromatographic purification of the crude product was done using methanol (2%) in ethyl acetate as eluant to get the product (**21**) (0.18 g, 33.1%), mp 160–61 °C. R_f 0.75 (1% MeOH in EtOAc). IR (KBr): 3395, 1654, 1596, 1531, 1484, 1245 and 1192 cm^{-1}. ^1H NMR: 2.45 (s, 3H, CH_3), 3.57 (b, 8H, CH_2), 6.65–6.68 (m, 1H, Ar-H), 6.86–6.88 (d, 1H, *J* = 8.8 Hz, Ar-H), 7.20–7.22 (d, 1H, *J* = 7.2 Hz, Ar-H), 7.50 (s, 1H, Ar-H), 7.54–7.58 (m, 1H, Ar-H), 8.05 (s, 1H, N–*H*), 8.13–8.14 (d, 1H, *J* = 4.4 Hz, Ar-H), 8.66 (s, 1H, Ar-H), 8.82–8.84 (d, 1H, *J* = 7.2 Hz, Ar-H). MS: (*m/z*) 365 (M^++1).

4.1.31. *N*-(8-Methyl-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)-4-[(4-methoxyphenyl)]piperazine-1-carboxamide (22)

Compound (**22**) was prepared by reacting the compound (**6b**) (0.3 g, 0.149 mmol) with 4-(4-methoxyphenyl)piperazine (0.287 g, 0.149 mmol) as described above for compound (**7**). Chromatographic purification of the crude product using methanol (2%) in ethyl acetate as eluant offered the titled compound (**22**) (0.195 g, 33.2%), mp 134–35 °C. R_f 0.78 (1% MeOH in EtOAc). IR (KBr): 3393, 1648, 1530, 1483, 1241, 1188 and 1153 cm^{-1}. ^1H NMR: 2.44 (s, 3H, CH_3), 2.97–2.99 (t, 4H, J = 4.6 Hz, CH_2), 3.58–3.61 (t, 4H, J = 4.6 Hz, CH_2), 3.80 (s, 3H, O-CH_3), 6.87–7.00 (m, 4H, Ar-H), 7.19–7.22 (d, 1H, J = 7.4 Hz, Ar-H), 7.50 (s, 1H, Ar-H), 8.00 (s, 1H, N*H*), 8.66 (s, 1H, Ar-H), 8.82–8.84 (d, 1H, J = 7.4 Hz, Ar-H). MS: (*m/z*) 394 (M^++1).

4.1.32. *N*-(8-Methyl-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)-4-benzhydrylpiperazine-1-carboxamide (23)

The isocyanate derivative (**6b**) (0.3 g, 0.149 mmole) and benzhydrylpiperazine (0.376 g, 0.147 mmol) were reacted together as described above for compound (**7**). The crude product was purified by chromatographic purification using methanol (0.5%) in ethyl acetate as eluant to obtain the compound (**23**) (0.2 g, 29.5%), mp 233–35 °C. R_f 0.54 (EtOAc). IR (KBr): 3000, 1644, 1529, 1481, 1237 and 1186 cm^{-1}. ^1H NMR: 2.32–2.34 (t, 4H, J = 4.4 Hz, CH_2), 2.43 (s, 3H, CH_3), 3.46–3.48 (t, 4H, J = 4.4 Hz, CH_2), 4.34 (s, 1H, C*H*), 7.18–7.22 (m, 3H, Ar-H), 7.29–7.33 (m, 4H, Ar-H), 7.44–7.48 (m, 5H, Ar-H), 7.87 (s, 1H, N–*H*), 8.63 (s, 1H, Ar-H), 8.79–8.81 (d, 1H, J = 7.6 Hz, Ar-H). MS: (*m/z*) 454 (M^++1).

4.1.33. *N*-[(7-Chloro-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)-4-(pyridin-2-yl)piperazine]-1-carboxamide (24)

In analogy to compound (**7**), (**24**) was prepared by reacting **6c** (0.3 g, 0.135 mmol) with 4-(2-pyridyl)piperazine (0.221 g, 0.135 mmol). Chromatographic purification of the crude product using methanol (2%) in ethyl acetate as eluant offered the compound (**24**) (0.21 g, 40.3%), mp 189–90 °C. R_f 0.45 (10% MeOH in EtOAc). IR (KBr): 3397, 3088, 1729, 1643, 1531, 1482, 1243 and 1124 cm^{-1}. ^1H NMR: 3.57–3.58 (b, 8H, CH_2), 6.65–6.68 (m, 1H, Ar-H), 6.86–6.88 (d, 1H, J = 8.4 Hz, Ar-H), 7.54–7.58 (m, 1H, Ar-H), 7.69–7.71 (d, 1H, J = 9.4 Hz, Ar-H), 7.83–7.86 (dd, 1H, J = 2.2 Hz and J = 9.4 Hz, Ar-H), 8.13–8.14 (d, 1H, J = 3.2 Hz, Ar-H), 8.16 (s, 1H, N–*H*), 8.82 (s, 1H, Ar-H), 8.89–8.90 (d, 1H, J = 2.2 Hz, Ar-H). MS: (*m/z*) 385 (M^++1).

4.1.34. *N*-[(7-Chloro-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)-4-(4-methoxyphenyl) piperazine]-1-carboxamide (25)

With a similar procedure, compound (**6c**) (0.3 g, 0.135 mmol) was reacted with 4-(4-methoxyphenyl)piperazine (0.26 g, 0.135 mmol) as described previously for compound (7). Chromatographic purification of the crude product was done using methanol (2%) in ethyl acetate as eluant yielding the titled compound (**25**) (0.25 g, 44.72%), mp 163–64 °C. R_f 0.75 (10% MeOH in EtOAc). IR (KBr): 3393, 1656, 1626, 1525, 1483, 1347, 1236 and 1065 cm^{-1}. ^1H NMR: 2.97–2.99 (t, 4H, J = 4.2 Hz, CH_2), 3.60–3.61(t, 4H, J = 4.2 Hz, CH_2), 3.80 (s, 3H, O–CH_3), 6.87–6.93 (m, 2H, Ar-H), 6.95–7.00 (m, 2H, Ar-H), 7.69–7.71 (d, 1H, J = 9.6 Hz, Ar-H), 7.83–7.86 (t, 1H, J = 9.6 Hz, Ar-H), 8.13 (s, 1H, N–*H*), 8.82 (s, 1H, Ar-H), 8.90 (s, 1H, Ar-H). MS: (*m/z*) 414 (M^++1).

4.1.35. Benzyl (4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl) carbamate (26)

Compound (**6a**) (0.3 g, 16 mmol) and benzyl alcohol (0.173 g, 16 mmol) in toluene (25 ml) were refluxed for 6 h to get solid precipitate. The solid precipitate was filtered and washed with hexane and the crude product so obtained was purified by column chromatography using ethyl acetate (90%) in hexane as eluant to get the titled compound (**26**) (0.5 g, 61.1%), mp 158–60 °C (Lit.[20] 153–55 °C). R_f 0.6 (1% MeOH in EtOAc). IR (KBr): 3239, 1718, 1650, 1461, 1274, 1228, 1180, 1116 cm^{-1}. ^1H NMR: 5.17 (s, 2H, CH_2), 7.32–7.50 (m, 6H, Ar-H), 7.69–7.71 (d, 1H, J = 8.8 Hz, Ar-H), 7.84–7.89 (m, 1H, Ar-H), 8.70 (br s, 1H, Ar-H), 8.92–8.94 (d, 1H, J = 6.8 Hz, Ar-H), 9.01 (br s, 1H, N–*H*). MS: (*m/z*) 296 (M^++1).

4.1.36. 2-Pyridinylmethyl (4-oxo-4*H*-[1,2-*a*]pyrimidin-3-yl) carbamate (27)

Compound (**27**) was prepared by reacting (**6a**) (0.5 g, 0.26 mmol) with pyridin-2-methanol (0.175 g, 0.16 mmol) as described above for compound (**26**). The crude product was subjected to chromatographic purification using ethyl acetate (90%) in hexane as eluant affording the titled compound (**27**) (0.24 g, 50.5%), mp.178–80 °C. R_f 0.6 (1% MeOH in EtOAc). IR (KBr): 3245, 3070, 1713, 1666, 1549, 1477, 1234 and 1125 cm^{-1}. ^1H NMR: 5.22 (s, 2H, CH_2), 7.33–7.38 (m, 2H, Ar-H), 7.51–7.53 (d, 1H, J = 8.0 Hz, Ar-H), 7.69–7.72 (d, 1H, J = 8.8 Hz, Ar-H), 7.83–7.89 (m, 2H, Ar-H), 8.55–8.57 (d, 1H, J = 4.4 Hz, Ar-H), 8.72 (s, 1H, Ar-H), 8.94–8.95 (d, 1H, J = 7.2 Hz, Ar-H), 9.21 (b, 1H, N–*H*). MS: (*m/z*) 297 (M^++1).

4.1.37. Benzyl (8-methyl-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)carbamate (28)

Compound (**28**) was prepared by treating the isocyanate derivative (**6b**) (0.5 g, 0.26 mmol) with benzyl alcohol (0.161 g, 0.149 mmol) as described above for compound (**26**). Chromatographic purification of the crude product using ethyl acetate (90%) in hexane as eluant yielded the titled compound (**28**) (0.18 g, 39%), mp 168–70 °C (Lit.[20] 177–79 °C). R_f 0.7 (1% MeOH in EtOAc). IR (KBr): 3242, 1717, 1635, 1546, 1465, 1224 and 1056 cm^{-1}. ^1H NMR: 2.45 (s, 3H, CH_3), 5.15 (s, 2H, CH_2), 7.21–7.23 (d, 1H, J = 7.4 Hz, Ar-H), 7.31–7.44 (m, 5H, Ar-H), 7.52 (s, 1H, Ar-H), 8.60 (s, 1H, Ar-H), 8.83–8.85 (d, 1H, J = 7.4 Hz, Ar-H), 8.97 (b, 1H, N–*H*). MS: (*m/z*) 310 (M^++1).

4.1.38. 2-Pyridinylmethyl (8-methyl-4-oxo-4*H*-pyrido[1,2-*a*] pyrimidin-3-yl)carbamate (29)

In analogy to compound (**26**), (**6b**) (0.5 g, 0.26 mmol) was reacted with pyridine-2-methanol (0.163 g, 0.149 mmol). Chromatographic purification of the crude product using ethyl acetate (90%) in hexane as eluant offered the titled compound (**29**) (0.19 g, 41%), mp 180–82 °C. R_f 0.75 (1% MeOH in EtOAc). IR (KBr): 3429, 3247, 1721, 1667, 1644, 1553, 1480, 1244 and 1117 cm^{-1}. ^1H NMR ($CDCl_3$): 2.45 (s, 3H, CH_3), 5.20 (s, 2H, CH_2), 7.22–7.24 (d, 1H, J = 7.4 Hz, Ar-H), 7.33–7.36 (m, 1H, Ar-H), 7.50–7.53 (m, 2H, Ar-H), 7.83–7.87 (m, 1H, Ar-H), 8.55–8.56 (d, 1H, J = 4.0 Hz, Ar-H), 8.62 (s, 1H, Ar-H), 8.85–8.87 (d, 1H, J = 7.4 Hz, Ar-H), 9.14 (b, 1H, N–*H*). MS: (*m/z*) 311 (M^++1).

4.1.39. *N*-(3,4-Methylenedioxybenzyl)-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidine-3-carboxamide (30)

Compound (**4a**) (0.5 g, 0.26 mmol) was suspended in dichloromethane (50 ml) and added 3,4-methylenedioxybenzylamine (0.397 g, 0.26 mmol), the reaction mixture 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 above solution. 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 HCl, extracted using ethyl acetate (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 (80%) in hexane as eluant offered the compound (**30**) (0.3 g, 35.2%), mp 193–95 °C. R_f 0.6

(EtOAc). IR (KBr): 3260, 3071, 1665, 1627, 1490, 1434, 1246 and 1224 cm^{-1}. ^1H NMR: 4.46–4.48 (d, 2H, J = 6.0 Hz, CH_2), 5.98 (s, 2H, CH_2), 6.82–6.93 (m, 3H, Ar-H), 7.58–7.62 (m, 1H, Ar-H), 7.89–7.91 (d, 1H, J = 8.8 Hz, Ar-H), 8.17–8.22 (m, 1H, Ar-H), 9.05 (s, 1H, Ar-H), 9.17–9.19 (d, 1H, J = 7.6 Hz, Ar-H), 9.31–9.33 (t, 1H, J = 6.0 Hz, N–H). MS: (m/z) 324 (M^++1).

4.1.40. *N*-(4-Fluorobenzyl)-8-methyl-4-oxo-4*H*-pyrido[1,2-*a*] pyrimidine-3-carboxamide (31)

Compound (**31**) was prepared by treating (**4b**) (0.5 g, 0.26 mmol) with 4-fluorobenzylamine (0.306 g, 0.24 mmol) as described above for compound (**30**). Chromatographic purification of the crude product using ethyl acetate (90%) in hexane as eluant afforded the desired product (**31**) (0.15 g, 51.9%), mp 185–87 °C. R_f 0.68 (1% MeOH in EtOAc). IR (KBr): 3287, 3014, 1677, 1633, 1481, 1279, 1222 and 1146 cm^{-1}. ^1H NMR ($CDCl_3$): 2.58 (s, 3H, CH_3), 4.65–4.66 (d, 2H, J = 4.0 Hz, CH_2), 7.00–7.04 (m, 2H, Ar-H), 7.18–7.21 (d, 1H, Ar-H), 7.34–7.37 (m, 2H, Ar-H), 7.63 (s, 1H, Ar-H), 9.08–9.09 (d, 1H, J = 4.0 Hz, Ar-H), 9.33 (s, 1H, Ar-H), 9.35 (br s, 1H, N–H). MS: (m/z) 312 (M^++1).

4.1.41. *N*-(3,4-Methylenedioxybenzyl)-7-chloro-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidine-3-carboxamide (32)

With a similar procedure, (**4c**) (0.5 g, 0.22 mmol) was reacted with 3,4-methylenedioxybenzylamine (0.336 g, 0.22 mmol) as described previously for compound (**30**). Chromatographic purification of the crude product using ethyl acetate (90%) in hexane as eluant yielded the product (**32**) (0.4 g, 50.2%), mp 199–201 °C. R_f 0.4 (EtOAc). IR (KBr): 3300, 1672, 1490, 1434, 1248 and 1036 cm^{-1}. ^1H NMR: 4.46–4.48 (d, 2H, J = 6 Hz, CH_2), 5.98 (s, 2H, CH_2), 6.81–6.92 (m, 3H, Ar-H), 7.90–7.93 (d, 1H, J = 8.8 Hz, Ar-H), 8.24–8.26 (m, 1H, Ar-H), 9.04 (s, 1H, Ar-H), 9.16 (s, 1H, Ar-H), 9.25 (b, 1H, N–H). MS: (m/z) 358.3 (M^++1).

4.2. Falcipain-2. enzyme assay

The diluted soluble *Plasmodium falciparum* FP-2 (30 nM) was incubated for 10 min at room temperature in 100 mM sodium acetate, pH 5.5, 10 mM DTT, with a fixed different concentration of the compounds to be tested or the standard (E64). Compound solutions were prepared from stock in DMSO. After 10 min incubation, the substrate Z-Phe-Arg-AMC was added to a final concentration of 25 μM. The fluorescence intensity was monitored (excitation 355 nm; emission 460 nm) for 10 min at room temperature with a Synergy™ 4 Multi-Mode Microplate Reader (BioTek). The inhibition rate (%) is calculated using the given equation:

$$\%\text{Inhibition} = [1 - (F\ \text{test}/F\ \text{control})] \times 100$$

where (F test is the fluorescence intensity of the test compound, F control is the fluorescence intensity of the standard (E64). All values are the means of three independent determinations and the deviations are <10% of the mean value. The IC_{50} values were determined for those compounds only which showed 40% or more of enzyme inhibition at 10 μM.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.09.008.

References and notes

1. Okafor, E. E.; Amzat, J. *J. Hum. Ecol.* **2007**, *21*, 155.
2. Ettari, R.; Bova, F.; Zappala, M.; Grasso, S.; Micale, N. *Med. Res. Rev.* **2010**, *30*, 136.
3. Wood, C. S. *Curr. Anthropol.* **1975**, *16*, 93.
4. Snow, R. W.; Guerra, C. A.; Noor, A. M.; Myint, H. Y.; Hay, S. I. *Nature* **2005**, *434*, 214.
5. Rosenthal, P. J. *New Targ. Chemother Emerg. Infect. Dis.* **1998**, *4*, 49.
6. Liu, J.; Istvan, E. S.; Gluzman, I. Y.; Gross, J.; Golberg, D. E. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 8840.
7. Korde, R.; Bhardwaj, A.; Singh, R.; Srivastava, A.; Chauhan, V. S.; Bhatnagar, R. K.; Malhotra, P. A. *J. Med. Chem.* **2008**, *51*, 3116.
8. Nwaka, S.; Riopel, L.; Ubben, D.; Craft, J. C. *Trav. Med. Infect. Dis.* **2004**, *2*, 161.
9. Shenai, B. R.; Sijwali, P. S.; Singh, A.; Rosenthal, P. J. *J. Biol. Chem.* **2000**, *275*, 29000.
10. Zhu, J.; Chen, T.; Liu, J.; Ma, R.; Lu, W.; Huang, J.; Li, H.; Li, J.; Jiang, J. *Molecules* **2009**, *14*, 785.
11. Liu, Y.; Cui, K.; Lu, W.; Luo, W.; Wang, J.; Huang, J.; Guo, C. *Molecules* **2011**, *16*, 4527.
12. Lesher, G.Y.; Schodack, N. Y. US Patent 3,907,798, **1975**.
13. Abass, M.; Ismail, M. M.; Abdel-Monem, W. R.; Mayas, A. S. *J. Serb. Chem. Soc.* **2010**, *75*, 11.
14. Li, R.; Kenyon, G. L.; Cohen, F. E.; Chen, X.; Gong, B.; Dominguez, J. N.; Davidson, E.; Kurzban, G.; Miller, R. E.; Nuzum, E. O.; Rosenthal, P. J.; McKerrow, J. H. *J. Med. Chem.* **1995**, *38*, 5031.
15. Klayman, D. L.; Bartosevich, J. F.; Griffin, T. S.; Mason, C. J.; Scovill, J. P. *J. Med. Chem.* **1979**, *22*, 855.
16. Lappin, G. R. *J. Am. Chem. Soc* **1948**, *70*, 3348.
17. Gannon, W. F.; Steck, E. A. *J. Org. Chem.* **1962**, *27*, 4137.
18. Nishigaki, S.; Ichiba, M.; Shinomura, K.; Yoneda, F. *J. Het. Chem.* **1971**, *8*(5), 759.
19. Shur, M.; Israelstam, S. S. *J. Org. Chem.* **1968**, *33*(8), 3015.
20. Simunek, P. *Heterocycles* **2008**, *75*, 2477.